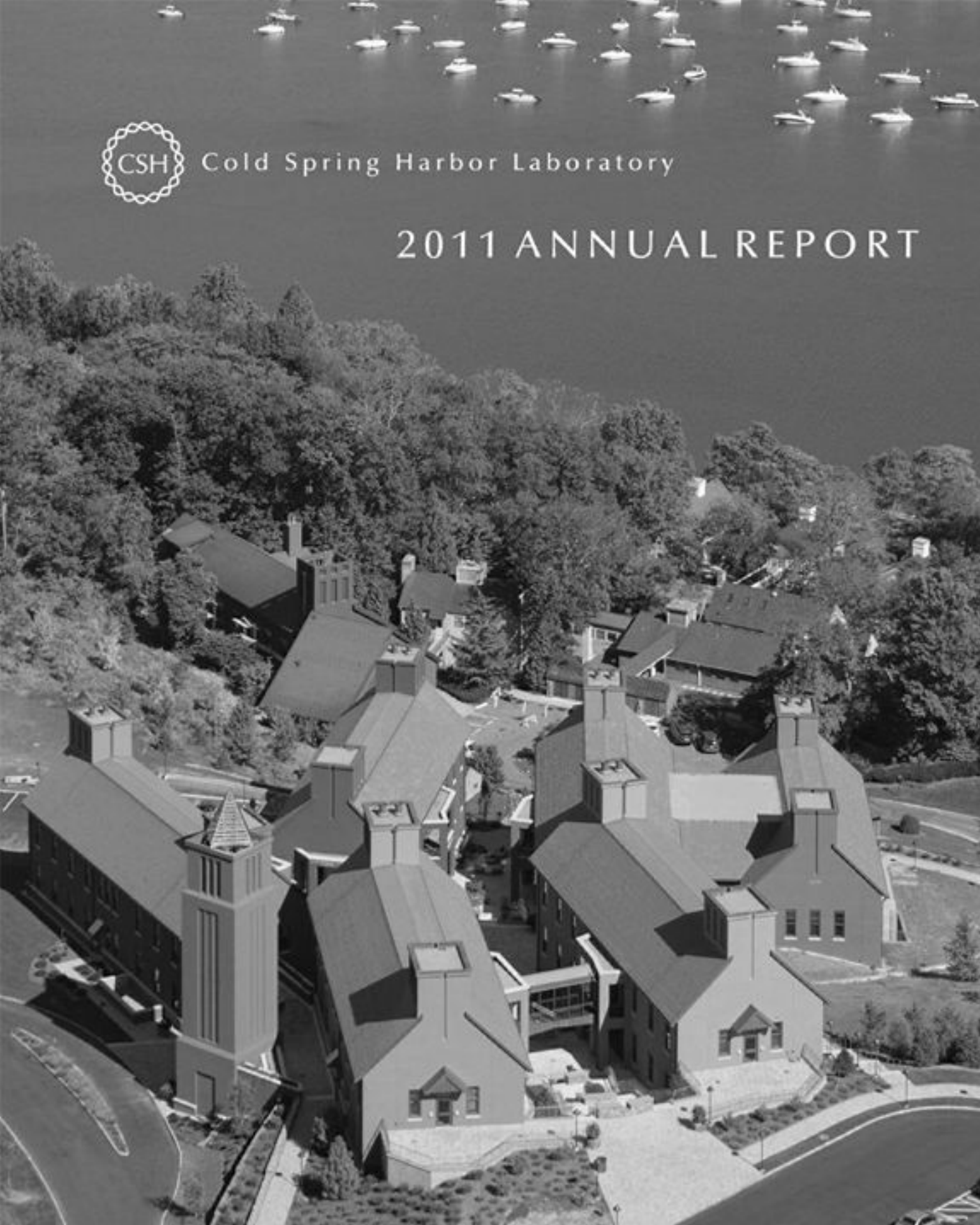
CSH Cold Spring Harbor Laboratory

2011 ANNUAL REPORT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Evelyn H. Lauder (1936–2011)

On hearing of her passing on November 12, 2011, Mike Wigler described her so compellingly: "She exemplified the best in human nature—grace and passion, intelligence and commitment, compassion and effectiveness, humor and seriousness of purpose." Evelyn H. Lauder was a remarkable woman.

At the Laboratory, she made her mark funding Mike's groundbreaking breast cancer research. Her relationship with him began in 1998, when he received his first grant from the Breast Cancer Research Foundation (BCRF). It was thanks to Larry Norton, who has directed the BCRF's scientific activities from the start, that Evelyn's organization was introduced to Mike and Cold Spring Harbor Laboratory. Our first BCRF grant was in 1998, including funds toward the purchase of microarray equipment to support collaboration between CSHL and Memorial Sloan-Kettering Cancer Center.

The evening before BCRF's fall 1998 research symposium, Liz and I were invited along with Mike and Deedee for dinner at Evelyn and Leonard's spacious Fifth Avenue apartment, whose more public walls were dominated by wonderful cubist-period paintings by Pablo Picasso. The next spring, in May, Evelyn, along with BCRF donor Lillian Goldman, Larry Norton, and BCRF Executive Director Susan Hirschorn, visited CSHL to view the equipment. A plaque naming the Lillian Goldman DNA Microarray Facility was presented. During the last 14 years, CSHL has benefited from nearly $4 million in funding from the BCRF.

At a late November 2002 fundraising evening, Liz and I, charmed by Evelyn's aerial photos of green Caribbean hills, quickly chose *Flying to Sasua* to hang in our recently purchased New York City apartment. Soon after, Evelyn graciously let us elect her as one of our Honorary Trustees. When she afterward dined with us, Evelyn saw how well the picture fitted in with the largely European paintings and drawings that we had collected over the then more than 35 years of our marriage.

When celebrating 50 years (1953–2003) of the Double Helix on DNA Day (April 25), we lit our 1904 Carnegie Building in pink lights to further raise awareness of breast cancer. After we initiated the Double Helix Medals, we honored Evelyn at a Mandarin Oriental celebratory dinner in November 2010 together with Mary-Claire King, who mapped *BRCA1*, the first human breast cancer gene. Also honored that evening was John Nash, whose profound idea on game theory revealed his most "beautiful mind."

Upon the occasion of my receiving the Aspen Institute's Public Service Award in the summer of 2007, I visited Evelyn and Leonard's rustic, no-frills home in the center of Aspen. A month before, I was alarmed to learn that cancer had reentered Evelyn's life. In her reply to my note of concern, she wrote back that her doctors had again given her a good prognosis. Battling ovarian cancer, how-

ever, is a much greater challenge than initially overcoming most breast cancers. Although her treatment soon led to her being symptom-free, only four years were to pass before her cancer came back with such ferocity that she lived only several more months.

Evelyn's caring smile and genuine empathy for the less fortunate are already sorely missed. Her memory will be best honored if the cancer world, today powerfully armed with the many fruits of the Human Genome Project, moves quickly into high gear against "incurable" late-stage breast and ovarian cancers. Skilled medicinal chemists need to develop innovative drugs that specifically target the molecular "Achilles' heels" of cancers that inevitably arise when normal cells become oncogenetically programmed for uncontrolled growth and cell division. Once we cross the starting line, no more than a decade of intelligent passion may be required. The sooner we start, the sooner we will win!

James D. Watson

PRESIDENT'S REPORT

Research directions at Cold Spring Harbor Laboratory Cold Spring Harbor Laboratory follow an ever-changing landscape that is influenced by the intellect of our scientists, by the evolution of scientific problems that can be addressed by current or newly developed technologies, and by a dynamic institutional view that promotes collaborative research and a focus on specific problems that are of collective interest. The neuroscience program at the Laboratory is a prime example of how three different areas of research have morphed together to contribute to major new insights into cognition and cognitive disorders.

A fall 2011 paper published in the *Proceedings of the National Academy of Sciences* from the laboratories of Alea Mills and Michael Wigler included Internet links to three rather grainy time-lapse videos. Each is a few minutes in length and allows us to peer into the cages of several mice simultaneously as they go about their activities. These videos demonstrate something potentially significant about autism genetics. The story behind the research suggests something equally interesting about the way in which we do science at Cold Spring Harbor Laboratory and about the evolution of our neuroscience program.

Mills' team had worked for several years to breed mice with a specific genetic defect that mimics one seen in certain children with autism. Called a copy-number variation (CNV), the defect is a deleted region on chromosome 16, referred to as 16p11.2. Its occurrence in autism, reported by Mike Wigler in 2007, has since been characterized as one of the most common spontaneous genetic alterations, yet it only affects less than 1% of children who have been classified with autism spectrum disorder (ASD). But what is its biological significance? CNVs, which are missing or extra segments of DNA, sometimes occur in regions that encode our genes. The 16p11.2 region contains 27 genes. One of the questions that the Mills and Wigler teams wanted to answer was if a mouse is missing this segment of chromosome 16, or has an extra copy of it, is its behavior affected—and if so, how?

The team did indeed observe behavioral changes, most prominently in mice lacking one of two copies of the 16p11.2 region that are present in most people. A normal mouse—or person—should have two copies, one inherited from each parent. Importantly, the abnormal mouse behaviors seen in the videos resemble some behavioral abnormalities seen in children with autism. In one video clip, a normal mouse quickly learns to climb to the highest point in her cage, while her sibling, bred with the 16p11.2 deletion, is observed during the same time interval to make almost no progress in her climbing ability. Another of the videos shows a mouse with the "autism" deletion unable during an extended interval—unlike her normal sibling—to figure out how to descend from the ceiling of her cage. A third clip shows a mouse with the 16p11.2 deletion displaying repetitive, stereotypical behavior in climbing down from a raised platform, a behavior not observed in healthy mice.

These experiments and others that they have set in motion serve to illustrate the power of a three-pronged approach to neuroscience that we have taken at the Laboratory. The three prongs, broadly described, are human genetics, cognition, and connectivity. For some years, these have tended to run in parallel. The success in creating an autism mouse model is notable because it demonstrates how the three areas of concentration are complementary, mutually informing and enabling, and ultimately integrative.

Neuroscience is one of the most dynamic fields in biology today. It has come a long way since one of its antecedents, called biophysics, was a subject of research at Cold Spring Harbor in the decade before the Second World War. Our first advanced courses on the subject, which commenced in the early 1970s with critical support from the Sloan Foundation and advice from John Nicholls, Eric Kandel, David Van Essen, Reg Kelly, and others, coincided with the installation of teaching labs in the old "Animal House" research building, a 1912-built laboratory now named for Barbara McClintock.

Not until the late 1970s did the Laboratory hire its first staff neurobiologists, Birgit Zipser and Ron McKay, who were joined in 1980 by then University of California at San Francisco postdoc and

future Massachusetts Institute of Technology (MIT) president Susan Hockfield. Their collaborations soon generated insights into the development and organization of the mammalian nervous system. But, alas, these forays into the emerging field of what the 1983 CSHL Symposium called "Molecular Neurobiology" were not sustained because the Laboratory lacked year-round space for neuroscience experimentation. It was a missed opportunity, but one decisively addressed by Jim Watson and an ad hoc committee of the Board of Trustees. In 1985, they set the Laboratory on a course that would result in a full and lasting commitment to neuroscience and in the construction of a major state-of-the-art laboratory. Named for Arnold and Mabel Beckman, it would enable CSHL to initiate year-round neuroscience research in the Laboratory's centennial year, 1990.

The current era of neuroscience investigation at Cold Spring Harbor dates from this time, over 20 years ago. An early focus on the molecular basis of learning and memory led, in time, to studies pertaining to neural dysfunction in people—to memory loss and, separately, to neurofibromatosis (NF), an inherited illness in which tumors form in nerve tissue. These studies, initially focusing on the fruit fly *Drosophila*, enabled our new neuroscientists to include a genetic approach to understanding the brain, taking advantage of the rich genetics legacy and research at CSHL. Studies were also begun to address questions pertaining to synaptic development and function.

In 1996, we recognized that still more space was needed to accommodate our growing neuroscience research effort, and we began to plan construction of a new facility that would be devoted, in part, to new techniques of imaging neurons and their functions in living animals. The centerpiece of the Nancy and Edwin Marks Laboratory was the Advanced Neuroscience Imaging Facility, in which Karel Svoboda collaborated with Roberto Malinow and Zach Mainen to introduce a powerful new tool called two-photon excitation laser-scanning microscopy to CSHL and, through our advanced laboratory courses, to the rest of the neuroscience community. This technology enabled our scientists to obtain images of individual neurons and synapses during sensory processing in live animals, something not possible previously. By October 1999, when the Marks building was dedicated, our neuroscience program had begun to explore the brain and nervous system in the context of each of the themes that I have mentioned—human genetics, cognition, and connectivity. For example, Dimitri (Mitya) Chklovskii studied in the worm *Caenorhabditis elegans* the reconstructed wiring diagram of the 279 nonpharyngeal neurons, a subset of the worm's 302 neurons, to reveal principles about how neuronal circuits deviate from optimal wiring and connection solutions. However, with the departure of a number of our neuroscientists to the newly established Janelia Farm Research Campus of the Howard Hughes Medical Institute, and elsewhere, we shifted our focus to understanding cognition and cognitive dysfunction, linking human genetics to behavioral neuroscience in a program developed in part by Tony Zador.

Zador, with Adam Kepecs and former colleague Zach Mainen, introduced the study of cognition in rodents as a powerful system for understanding complex cortical events in the brain. Their studies enabled them to investigate how rodents, and ultimately humans, use external stimuli to make decisions and pay attention, and use working memory for cognitive tasks. Later joined by Anne Churchland and Florin Albeanu, they are investigating how external stimuli such as odors and sound work their way through the initial sensing regions of the brain to be coded in working and long-term memory that in turn influences behavior. Today, our neuroscientists—who have expanded their activities beyond Beckman and Marks to the new Hillside Laboratory buildings—are able to use sophisticated techniques called optogenetics, a method invented at Stanford University that enables individual neurons to be switched on and off with beams of colored laser light delivered painlessly via fiber-optic threads into the cortex of behaving rodents. Zador, Kepecs, and Albeanu, along with Stephen Shea and Glenn Turner, have mobilized this and other advanced recording technologies to explore how incoming sensory signals are processed in successively more complex layers of neural anatomy, from the sensory epithelia in the nose to the olfactory bulb and olfactory cortex in rodents, and analogous structures in fruit flies. Shea is studying the way in which the emotional salience of an experience—say, an encounter with a potential mate—is encoded, along that path leading from

olfactory receptors to the brain's olfactory cortex, where decisions are made based on processed signals. Some decisions, it is believed, are innate, whereas others, such as these, are forged in response to experience or environmental cues. Tony Zador, who heads our Swartz Center for Computational Neuroscience, established computational analysis of brain circuits as an important part of this work. Zador uses the rodent auditory system as his model in work that seeks to better understand how neural dysfunction can produce the kinds of deficits in social interaction and attention that we see in disorders such as autism and schizophrenia.

Bo Li's research places him at the nexus of behavior and another of the three major themes of neuroscience at the Laboratory: connectivity. When they study connectivity, our scientists broach the immense question of the mammalian brain's architecture and the relationship of structure to function. This can be studied at multiple levels of detail: from the structure of the synapses at which individual neurons communicate, to circuits and networks composed of ever-shifting ensembles of interconnected nerve cells, to entire regions of the brain in which specific kinds of tasks are handled by the neural network. In 2011, Bo Li and Fritz Henn collaborated to discover that neurons in a tiny area in the central brain called the lateral habenula (LHb) are hyperactive in depressed rodents. They found that dysfunction in this one area creates imbalances in other areas of the brain to which it sends neural projections. Understanding the nature of these interrelationships holds a potential key to new ways of treating depression, and, as a result of these studies, Fritz Henn has begun to translate these results into a clinical trial focusing on deep-brain stimulation in the LHb of patients with otherwise untreatable chronic depression.

Connectivity in its neuroscience context is the theme of many different CSHL studies at the cellular level. Linda Van Aelst, an expert on signal transduction, has complemented her work on brain cancer in children with research on how mutations in signaling molecules in the Ras and Rho families of proteins are implicated in various brain disorders. Linda has extensively studied functional implications of mutations of the *oligophrenin-1* gene in several neuropathologies, including X-chromosome-linked mental retardation, and has found that the gene is essential in activity-dependent maturation of synapses and the ability of an existing synapse to change, commonly called plasticity. Linda's interest in neurodevelopment is complemented by the work of Grigori Enikolopov and Alex Koulakov, who study the role played by stem cells during brain development as well as in the adult brain. In 2011, they found that each adult brain stem cell in mice is used only once to amplify into many neurons and hence is disposable, as opposed to stem cells in the blood or gut that self-renew many times during

their life span. Whether stem cells that have been expended can be enticed to revert to their former identity and be recruited back into the stem cell pool is a question that Enikolopov's team is now pursuing. Hiro Furukawa's research concerns another aspect of nerve cell biology, that of proteins that lie on the surface of individual nerve cells. He studies the structure of the immense multiunit protein that forms NMDARs (*N*-methyl-D-aspartate receptors) that control the strength of connections between neurons and thereby have a central role in learning and memory. Membrane receptors, the place where neurotransmitter molecules "dock" with nerve cells, are at the "front end" of cell-signaling networks and are of great interest as targets for drug discovery in diseases such as Alzheimer's and in cognitive disorders such as autism and depression. This year, Hiro's team discovered and mapped a new regulatory site in a class of NMDARs, progress that now opens the way to the development of a potentially new class of drugs to modulate the receptor.

By a very rough estimate, the human brain contains 100 billion neurons, but they are certainly not all the same cell type. Some are excitatory and activate circuits, and others are inhibitory and modulate neuronal networks. Josh Huang is studying a class of inhibitory neurons that are divided into about several dozen different types. He is interested in neural development and has invested much time and effort in creating a powerful new resource for the entire neuroscience community. His team has developed 20 different mouse lines, each of which is engineered to express markers of specific cell types. They recently demonstrated the exquisite specificity of this technology by imaging the main inhibitory nerve cell type in the mammalian brain, so-called GABA cells. In a fascinating series of images, Josh's team showed a particular subset of GABA cells as they moved from their place of birth in the mouse brain through various stages of the developmental process. We can see them migrate into the cortex and take up positions at which they forge connections with one another and with excitatory neurons. This type of plasticity underlies much of how our brain develops and responds to learning and memory of new tasks.

The way in which the brain is wired can be studied at many levels, from how individual neurons are connected to one another, to how groups of neurons form networks of cells that "compute" information, to how the small networks are connected to one another to form regions of the brain that are linked to recognizable tasks such as vision, motor control, and hearing. Finally, our brain is composed of large functional regions that communicate with one another and coordinate all sensory input and behavioral responses. Using funding from a National Institutes of Health TR-01 grant and funds from the Allen Institute for Brain Science, both awarded to advance potentially "transformational science," Tony Zador is developing a novel, high-throughput method to probe the connectivity of neural circuits at the level of individual neurons in mice—called the "connectome." He is collaborating with molecular biologist Greg Hannon to use very innovative technologies that allow molecular codes to be passed from one neuron to another in the brain, later capturing how the information is linked using powerful DNA sequencing technologies. The potential for understanding how networks of neurons are wired is enormous. Josh Dubnau, another TR-01 grant recipient, is approaching neural connections from an entirely different angle. He seeks to assemble a comprehensive store of information about how RNAs are translated into proteins needed for the assembly of synapses. He and his team are focusing initially on spatial and temporal control of translation in the context of fragile-X syndrome, an autism spectrum disorder in which the process goes awry.

On a different scale, two exciting imaging technologies have recently been developed at CSHL and are beginning to yield valuable data regarding brain structure and connectivity. Partha Mitra is progressing in an effort backed by a third TR-01 grant to map the mouse brain at what he defines as the "mesoscopic" level. Partha's project is focused on identifying the nature of the connections within and between different brain regions and eventually integrating the data with the Allen Brain Atlas, which has proven to be a valuable map of genes that are expressed in different regions of the mouse brain. Partha and his team have built a robotic laboratory that enables the analysis of many mouse brains and the storage of very large data sets for computational analysis.

An ultimate scale of understanding brain activity and connectivity is at the cellular level. Pavel Osten and his team have built an impressive automated system dedicated to systematic brain connectivity and functional studies. By automating and standardizing the process by which brain samples are divided into very thin sections and then imaged sequentially with precise spatial orientation by high-resolution microscopes, Pavel has opened the door to making whole-brain mapping at the cellular level more routine. In essence, each neuron in the brain has a gene that is turned on when the neuron has been recently active, expressing a green fluorescent protein (GFP) that can be imaged. It is possible to image all of the brain cells that "light up" after the mouse has a new experience or receives a drug that affects brain activity. This technology, along with the technology developed by Mitra, will enable an analysis of the connectivity and activity within the brains of mice bred with the autism 16p11.2 CNV. This application of brain imaging of mice with genetic alterations that mimic those in patients with Alzheimer's disease, autism, bipolar disorder, depression, and schizophrenia is one aspect of the "integration" of science that is characteristic of research at CSHL. It makes perfect sense, given a credible animal model of a brain disorder, to explore at the level of both structure and function how the brain in an abnormal phenotype differs from that in a healthy control. This should provide unique and, we hope, powerful insights into both disease causation and new therapeutic approaches.

The integration of connectivity and behavioral studies is only two-thirds of the story of integration. The final third concerns human genetics but in many ways is a key starting point for understanding how the brain functions. It is the part with the longest and richest history at Cold Spring Harbor—one that takes us back to our institutional roots and forward to current and future research. In what I consider a classic instance of research in the CSHL style, Alea Mills—a geneticist—became involved in an important neuroscience project because of a technique that she had previously invented called chromosome engineering. Some years ago, she demonstrated its utility in solving a decades-old mystery about a section of human chromosome 1 that is frequently deleted in neural, epithelial, and hematopoietic cancers.

It had long been clear that this region of the chromosome harbored an important tumor-suppressor gene. But the region was large and, like the 16p11.2 region in autism, was known to contain many genes. Which was the tumor suppressor? Mills and colleagues created mice with gains and losses of genomic regions that corresponded to the deleted region on chromosome 1. They confirmed that loss of the region predisposes to cancer, whereas gain of the region results in excessive tumor suppression. Ultimately, they identified *Chd5* as the tumor suppressor within the region and determined that the protein that it encoded was a remodeler of chromatin that regulates an extensive tumor-suppressive network. Thus, a technology developed to study cancer genetics made possible the engineering of the "autism mouse."

Another thread of current neuroscience research at CSHL can be traced to cancer research and goes back even further than chromosome engineering. During the 1980s, as Michael Wigler and his group studied the genetics of breast cancer, Mike became interested in comparing a woman's normal DNA to the DNA found in her tumor. In collaboration with former colleague Nikolai Lisitsyn, he invented a technique called representational difference analysis (RDA), followed by a second-generation method called representational oligonucleotide microarray analysis (ROMA) developed with Rob Lucito, to perform such comparisons. This evolved over several years into successive variants of the technique, including the recently developed ability to sequence the genome of a single cell, enabling comparisons to be made on a whole-genome scale. By collecting DNA from hundreds of women with breast cancer, Wigler and collaborators were able to build an extensive DNA database that, among other things, also enabled them to compare the DNA readouts of the noncancer, or normal, DNA from each woman. But unexpectedly, an important discovery followed that revised our notion of what normal means. Every supposedly normal sample contained a large number of DNA variations relative to the standard human reference genome. Each person was significantly different because they harbored gene CNVs. This led to the realization that CNVs were not a rare and exotic form of genome mutation but instead were present in every individual.

Wigler pursued an inspired hunch that some common genetic disorders—such as autism and schizophrenia—might be traceable to CNVs that occurred spontaneously, that is, not present in either parent of an affected individual. In subsequent work, this hypothesis was confirmed for both disorders. In autism especially, it became clear that spontaneous CNVs accounted for a large fraction of cases. With generous support from the Simons Foundation, a large sample of families with an autistic child was assembled. Analysis of the genomes of the affected child compared with that of the parents and sometimes an unaffected sibling enabled the identification of hundreds of regions in the human genome associated with autism. The surprising fact that we all have CNVs, coupled with the Wigler lab's invention of the technological means to identify them across the genome, was a major step along the path toward understanding the cause of the disorder and toward the eventual use of chromosome engineering to create a mouse model of autism.

Mike Wigler continues to explore the genetics of neuro-psychiatric disorders in humans. He and collaborators have also made a major contribution to describing the role of both spontaneous and inherited CNVs in schizophrenia. Another of our senior scientists, W. Richard McCombie, shares this interest in the human genetics of brain disorders. Dick heads the Laboratory's Stanley Institute for Cognitive Genomics and is sequencing patients with schizophrenia, bipolar disorder, and depression. The collaboration between Wigler and McCombie has enabled the genetic analysis of autism to proceed to identifying rare single-gene mutations.

The three paths of research in neuroscience—human genetics, connectivity, and the study of cognition—are rapidly converging. Furthermore, the Laboratory's recent investment in building a Quantitative Biology program is infusing our neuroscience program with a new source of intellectual vibrancy. In 2011, Ivan Iossifov, a CSHL Quantitative Biology Fellow, along with Michael Ronemus, Dan Levy, and Sarah Gilman in the Wigler lab, collaborated with Dennis Vitkup at Columbia University to undertake a novel quantitative study of CNVs in autism spectrum disorder. One of their published papers addressed a question that is now being asked in the Mills–Wigler collaboration about their autism mouse model: Which genes within a CNV of interest are actually involved in disease pathology?

Iossifov, Vitkup, and colleagues used a novel form of statistical analysis to identify the large biological network of genes affected by rare spontaneous CNVs in autism. They found that this network was strongly related to genes previously implicated in studies of autism and intellectual disability. And they confirmed that the genes in question relate primarily to the development of synapses as well as to the targeting of axonal fibers and the ability of young neurons to migrate. All are essential aspects of brain development. In more recent studies to be published in 2012, Iossifov, Ronemus, Levy, McCombie, Wigler, and collaborators have sequenced the genomes of families with a child with autism and have made some remarkable discoveries. First, the age of the father is a significant contributor to the occurrence of spontaneous gene mutations that are linked to autism. Second, a large percentage of the genes found to be causative of autism fall into a network of genes whose RNAs are regulated by fragile-X mental retardation protein (FMRP). Fragile-X syndrome is a disorder on the spectrum of autism disorders and is caused by a mutation in a single gene. By studying how the defect in the *FMRP* gene alters synaptic activity in the brain, Mark Bear and his colleagues at MIT have developed a drug that targets the neuronal mGluR5 protein that reverses the effect of the fragile-X syndrome mutation in mice. The drug is now in clinical trials in children with fragile-X syndrome. The fact that a disorder on the autism spectrum can be reversed is remarkable in itself, but the observations that genes that cause autism overlap with FMRP and fragile-X syndrome offer the possibility that more common forms of autism, or at least a subset of autism, might be treatable. This finding suggests how the fruitful integration of nominally distinct research approaches is providing major insight into how the brain functions and offers hope that some form of treatment may be on the horizon. I look forward to further substantial progress in our neuroscience research that is as exciting as it has been during the past 15 years.

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

Highlights of the Year

Research

In summing up the year's achievements in the annual "State of the Lab" presentation to Laboratory staff, I characterized 2011 as one in which research productivity was "truly exceptional." In fact, Cold Spring Harbor Laboratory remains at the very top of the list of scientific research institutions worldwide for its impact, as measured by science publisher Thomson Reuters. During the last decade, research papers in molecular biology and genetics published by CSHL investigators have been cited, on average, more frequently than those published by scientists in comparable institutions.

These statistics say something important about CSHL scientists beyond the general reputation of the institution, which has long been stellar. They tell us that work coming out of their laboratories is consistently important to their colleagues at other institutions, who trust, cite, and build upon their data. Notable about research at Cold Spring Harbor Laboratory is the highly collaborative nature of the research, which is focused on a few areas, including cancer, neuroscience, plant biology, and quantitative biology. Research results in 2011, highlighted below, indicate how studies in basic science, when pursued consistently over time, provide the basis for practical advances in medicine. Here is a sampling of a few of the many important results obtained by CSHL scientists in 2011.

Unconventional Hunt for Cancer Targets Yields a Drug Candidate of Unusual Potential

C. Vakoc

Using an unconventional approach to discover new targets for anticancer drugs, a team led by CSHL Fellow (and now Assistant Professor) Christopher Vakoc along with Professor Scott Lowe pinpointed a protein called Brd4 as a novel target in the treatment of acute myeloid leukemia (AML). AML is an aggressive blood cancer that is incurable in about 70% of patients. In an early success for CSHL's new Cancer Therapeutics Initiative, Vakoc, Lowe, and their colleagues used a sophisticated screening method based on RNA interference (RNAi), developed in Greg Hannon's laboratory, to identify Brd4, a protein that helps regulate gene expression by reading chemical tags that attach to chromatin, the material in which the genetic material is bundled. They used short-hairpin RNAs to disable the gene that encodes Brd4 in mouse models of AML developed by Lowe, halting progression of the disease and bringing about significant remissions. In collaboration with physician and chemist Dr. James Bradner at the Dana-Farber Cancer Institute, the team was able to reproduce these antileukemic effects using a small-molecule drug called JQ1, previously characterized by Bradner to specifically inhibit the Brd4 protein. That drug is now being optimized for testing in human patients with AML, in trials that should begin within the next 18 months. Because Brd4 is a link in an intracellular signaling pathway that includes the major human cancer gene c-*Myc*, there is a chance that if successful, JQ1 or its analogs may be effective in treatment of other human cancer types in which c-*Myc* activation has an important role.

New Therapeutic Target for Liver Cancer and a Predictive Biomarker

S. Powers

In another research effort that highlights "next-gen" approaches in cancer research, Associate Professor Scott Powers and colleagues identified a strategy for targeted molecular therapy in liver cancer, which currently has limited treatment options and one of the worst one-year survival rates of any cancer type. Their experiments reveal that up to 15% of liver tumors are driven by the hyperactivity of a gene called *FGF19*, which is involved in various normal biological processes such as cell growth and tissue repair. Powers' collaborator, Dr. Dorothy French at Genentech, had previously developed a potent antibody that blocks the activity of the protein produced by the *FGF19* gene. Experiments showed that shutting down its activity with the antibody inhibited tumor growth. This work therefore provides

not only a potential treatment but also a biomarker—*FGF19* gene amplification—to predict whether treatment with the antibody is going to be effective. The research represents a new chapter in efforts to expand the current goals of large-scale cancer genome projects in which whole-genome sequencing of cancer genomes is pinpointing new therapeutic strategies and drug targets.

Relationship between Two Genes Mutated in Prostate Cancer Can Dictate Outcome and Aid Treatment Decisions

Prostate cancer is common, but only about 15% of newly diagnosed cancers progress to a malignant and lethal form. Identifying those patients who might progress is of high importance. Research by Associate Professor Lloyd Trotman and colleagues addressed this issue when they set out to discover what kind of gene deletions work together with the loss of a tumor suppressor gene called *PTEN* to trigger prostate cancer. The PTEN protein normally removes phosphate molecules from its molecular targets and tempers prostate cell proliferation by preventing activation of a cancer-causing gene called *AKT*. Focusing on another phosphatase, PHLPP1, that was recently identified as a deactivator of *AKT*, Trotman's team showed that the *PHLPP1* gene also functions as a suppressor of prostate tumors. Mice that lacked both copies of *Phlpp1* developed a premalignant form of prostate cancer. The team found that monitoring the activity of the two genes—*PTEN* and *PHLPP1*—in patients following prostate surgery can predict whether a patient is on a path to develop dangerous dual deletions and thus relapse following initial hormone therapy. This information could help identify the best patients for clinical trials testing a new class of prostate cancer drugs that inhibit the *AKT* pathway and could also influence which inhibitors of this type are used and when.

L. Trotman

New Method Reveals How Tumors Evolve and Spread

A new analytic method devised by Professor Michael Wigler, Research Professor James Hicks, and colleagues features a process called single-cell sequencing that enables accurate quantification of gene copy number within the DNA of a single cell. Gene copy number refers to the number of each gene in the cell, and normally it should be two (one inherited from each parent). In cancer, portions of the genome are amplified or deleted, giving rise to extra or missing copies of key genes and interfering with mechanisms that normally control cell growth and proliferation. Single-cell sequencing, the latest in a long line of technical innovations by the Wigler team, represents a major advance in our ability to understand how tumors evolve, uncovering the genetic complexity within a single tumor. The insights offered by this method have relied heavily on research within the new program of quantitative biology at CSHL that has expanded in the last several years. The analysis of breast cancers using single-cell sequencing methods suggests that tumors may not evolve gradually, but rather in punctuated bursts. It is a finding that has already shed new light on the process of tumor growth and metastasis and should help in the development of new methods to clinically evaluate tumors.

Powerful Quantitative and Clinical Study on Autism Causation and Gender Skew

Dan Levy, Michael Ronemus, and others investigators in the Wigler lab joined with CSHL Quantitative Biology Fellow, now Assistant Professor, Ivan Iossifov to complete a clinically extensive and mathematically powerful study of autism spectrum disorder (ASD). Using a newly assembled patient and family sample population called the Simons Simplex Collection—which at the time included about 1000 families comprising both birth parents and in most cases two children, one with ASD and the other unaffected—the team affirmed prior results stressing the importance of noninherited, spontaneously occurring copy-number variations (CNVs) as causal factors in autism. The team was able to consistently resolve much smaller genomic irregularities than previously possible. This analysis revealed a significantly greater number of genome areas where spontaneous DNA deletions and duplications affected genes thought to contribute substantially to ASD. The team estimates a minimum of 250–300 places in the genome harboring CNVs can give rise to ASD. Most of the CNVs detected

M. Wigler

were only seen once and are classified as rare gene mutations. This is a potential source of confusion because these rare events, taken in total, are possibly the source of at least half of all autism and, based on Wigler's previous studies, contribute to inherited forms of autism.

Mice Engineered with Chromosome 16 Deletion Seen in Humans Display Autism-Like Behaviors

A. Mills

Professor Alea Mills, together with Mike Wigler and colleagues, achieved an important milestone in 2011, proving that one of the most common genetic alterations in autism—deletion of a 27-gene cluster on chromosome 16—causes autism-like features. By generating mouse models of autism using a technique known as chromosome engineering, the Mills team provided functional evidence that inheriting fewer copies of these genes leads to features resembling those commonly used to diagnose children with autism. The behavior of mice with the deletion contrasted with that of normal mice; they were hyperactive, had difficulty adapting to a new environment, suffered from sleeping deficits, and displayed restricted, repetitive behaviors. Mice engineered to bear human gene aberrations associated with autism will be invaluable in pinpointing the disorder's genetic basis and elucidating how these alterations affect the brain. They could also be used for inventing ways to diagnose children before they develop the full-blown syndrome and for designing clinical interventions.

Overlooked Brain Area Found to Be an Important Locus of Depression

The scientific importance of animal models was also shown in experiments performed by Assistant Professor Bo Li, Professor Fritz Henn, and colleagues at Brookhaven National Laboratory and the University of California, San Diego. By implanting electrodes into a tiny area of the rat brain called the lateral habenula (LHb), they were able to deliver an analog of deep brain stimulation (DBS). This had the effect of reversing depression-like symptoms in the rats. The team's results point to DBS in the LHb as a potential therapy for depression. DBS is already an important treatment method for Parkinson's disease.

Precise Structural Maps of NMDA Receptors Will Aid Drug Discovery

In the search for the mechanisms that give rise to depression, scientists have a variety of clues, one of which is dysfunction of receptors in the brain's neurons called NMDA (*N*-methyl-D-aspartate) receptors. These widely expressed receptors are large, multiunit proteins found at the membrane of a subset of excitatory neurons. The way in which a neurotransmitter binds to the receptor determines the strength of neuronal electrical excitability. In 2011, Associate Professor Hiro Furukawa and his team of structural biologists published the results of two important studies. In one, they mapped the precise shape of the subunit of the NMDA receptor that binds excitatory neurotransmitters (notably glutamate, the brain's most prevalent excitatory neurotransmitter). In a second study, they obtained a precise molecular map of the binding site for an allosteric inhibitor of an NMDA receptor subtype. This site—a docking port within the receptor—is important because it is a potential target for drugs that can modulate NMDA receptors. Problems in these receptors have been linked not

B. Li

F. Henn

H. Furukawa

only to depression, but also to a number of other important disorders including schizophrenia, Parkinson's, and Alzheimer's diseases, as well as stroke-related brain injuries.

Stem-Cell-Related Changes that May Contribute to Age-Related Cognitive Decline

Why do our brains produce fewer and fewer neurons as we age—a phenomenon thought to underlie the process of age-related cognitive decline? Blood stem cells go through many cycles of self-renewal followed by quiescence, and their numbers do not decline with age. But Associate Professor Grigori Enikolopov's group, together with Associate Professor Alex Koulakov, has found, in contrast, that adult stem cells in the brain remain quiescent for a prolonged time before they are activated. Then, they undergo a series of rapid divisions, giving rise to progeny that differentiate into neurons. After that, the stem cells abandon their "stemness" by differentiating into astrocytes, a type of nonneural "helper" cell. This implies that each adult brain stem cell is used only once and hence is disposable. This in turns raises a fascinating question: Does activating neuronal production too much—say, with the use of neurogenesis-enhancing drugs such as the antidepressant Prozac—exhaust the stem cell pool prematurely? The answer depends on how the production of new neurons has been induced, according to Enikolopov. Whereas Parkinson's disease and traumatic brain injury, which activate stem cells directly, may result in a depletion of the stem cell pool, therapeutic deep brain stimulation of certain brain areas, as well as Prozac and exercise, stimulates the downstream progeny of stem cells and increases production of new neurons while keeping the stem cell pool itself safe and intact.

Paths to New Drugs from Basic Research on Protein Tyrosine Phosphatases

Professor Nicholas Tonks and his team have discovered how targeting an enzyme called PTP1B—which Tonks discovered in 1988—can provide a path to the discovery of new drugs to fight two major scourges, diabetes and obesity. As the "founding member" of a superfamily of enzymes called protein tyrosine phosphatases (all of which remove phosphate groups from tyrosine residues in proteins), PTP1B is known to be an important player in the intracellular signaling pathway that regulates the response to insulin. In type 2 diabetes, insulin molecules dock at cells but the cellular mechanism that sends their signal does not work properly. This year, Tonks and his colleagues found that PTP1B is regulated by hydrogen sulfide (H_2S). Tonks demonstrated reversible control of PTP1B activity by H_2S in cells by the addition of a sulfur molecule to the active-site cysteine residue in the enzyme, thereby inhibiting the enzyme activity. They also discovered a new allosteric mechanism that regulates PTP1B activity, offering the possibility of developing small-molecule drugs that could modulate PTP1B activity. Tonks' team generated a specific type of antibody that, when expressed in cells, selectively recognized and stabilized an oxidized state of PTP1B. In that mode, its role in suppressing insulin signaling is inactivated, resulting in increased insulin signaling. This had the effect of enabling insulin to transmit its signal in an enhanced and sustained fashion. It suggests a new way of targeting PTP1B in therapeutic efforts to address insulin resistance that occurs in diabetes. But this pathway that influences metabolic signaling may also influence how primary breast cancer cells switch to metastatic tumor cells and invade other parts of the body. In two separate papers, Tonks' team identified and characterized a novel allosteric inhibitor of PTP1B that indicates a unique mode of therapeutic intervention in HER2-positive breast cancers, and, using RNAi screening to test the role of PTP family members in controlling migration and invasion in mammary epithelial cell models of breast cancer, they discovered that PTPN23, another phosphatase family member, is among the regulators of such invasion, exerting its effects by regulating the well-known tumor-promoting SRC protein. Their research indicates that inhibitors of SRC might be useful in treating patients with inactivating mutations in the *PTPN23* gene.

N. Tonks

How Plants Are Able to Overcome Environmental Challenges in Order to Thrive

If an animal gets too hot or too cold, or feels pangs of hunger or thirst, it tends to relocate—to where it is cooler or hotter, or to the nearest place where food or water can be found. But what can a plant

do under similar circumstances? Plants cannot change the climate and are unable to uproot themselves to move to a more favorable spot. Yet they do respond successfully to changes in environmental conditions in diverse ways, many of which involve modifications of the way they grow and develop. Professor David Jackson and his team at CSHL have now discovered at the genetic level how one vitally important species of grass plant—maize—responds to the challenge to growth posed by shade. Central to this work is the team's identification of the role played by a gene called *grassy tillers1*, or *gt1*, whose expression, they confirmed, is controlled by light signaling. Maize plants produce very few tillers, or lateral branches, at their base. Plants with profuse tillers do not tend to grow well in close proximity because their branches and leaves tend to throw any close neighbors into shade, thus limiting access to sunlight, their common prime energy source. By severely limiting its lateral branching, maize is able to redirect its energy to the primary shoot, which grows taller and escapes the shade. A fascinating sidelight: Without any knowledge of genes or genetics, ancient Mesoamericans domesticated maize by selecting for genes that suppressed tillering and thus encouraged maize to grow upward to the sky, rather than outward into the neighboring stalk. We now know one molecular mechanism of how the corn that was selected by these early Indians grows to yield tall plants.

D. Jackson

Cold Spring Harbor Laboratory Board of Trustees

At the core of the success of Cold Spring Harbor Laboratory are the philanthropic organizations and generous individuals who recognize the significance of our biomedical research and education programs to the community and society at large. Investments in CSHL are driving scientific and medical progress as well as economic development. With more than 1100 employees, the Laboratory is one of the largest employers in the region. Technologies developed at CSHL have sparked new companies, some of which have grown to be players in the global economy. One example is OSI Pharmaceuticals, which was acquired by Astellas Pharma, but importantly remains on Long Island at the Broad Hollow Bioscience Park. CSHL helped found Broad Hollow at SUNY Farmingdale as an incubator for life science start-ups. More broadly, a new science cluster that includes CSHL, Brookhaven National Laboratory, Hofstra University, North Shore–LIJ Health System, and Stony Brook University could become a formidable engine for economic growth. Combined, the institutions represent a major economic force on Long Island and in New York State.

With an eye to the future, CSHL this year became a founding member of the New York Genome Center (NYGC), a nonprofit organization leveraging the collaborative resources of 11 leading academic medical centers, research universities, and hospitals in the vicinity of New York City. This consortium will generate data essential for the advance of personalized medicine, accelerate the development of new diagnostics and treatments for human disease, and promote life science commercialization. It will complement the strong genome research center that is currently the only genome center in New York State and that has contributed to major genome sequencing projects and investigation of the causes of human disease and cognitive disorders.

CSHL's leadership role in developing and using genomic technologies will provide the NYGC with expertise that will facilitate the genomic analysis of patient samples. This in turn will expedite the identification of mutations that drive disease processes, as well as critical prognostic and diagnostic markers for disease.

I am thankful for the active involvement and support that we receive from the Board of Trustees. In 2011, the Board elected George Sard, President of Sard Verbinnen & Co. The Laboratory community mourned the loss of Honorary Trustee Evelyn Lauder. Her leadership of the Breast Cancer Research Foundation, which supports research conducted at the CSHL Cancer Center and numerous breast cancer research centers throughout the world, has been invaluable in raising awareness and funds for research and treatment.

A. Harmon and J.C. Nicholls, CSHL Board of Trustees Chairperson

With newly elected president Sandy Tytel, the CSHL Association is the institution's grassroots connection to the community. In its "Science Never Sleeps" campaign, the CSHLA this year helped raise a record $5.9 million. "Labapalooza" was a unique fundraiser featuring scientist rock bands and tastings from Long Island's best restaurants and enabled our talented scientists to display their musical flair.

The 10th annual "Women's Partnership for Science" event featured Pulitzer-Prize–winning *New York Times* journalist Amy Harmon, who presented "Targeting Cancer: A Dose of Hope." During the last decade, the Partnership has raised almost $700,000. This year's luncheon drew nearly 150 women, who gathered on the scenic lawn of Airslie House.

Research Faculty

G. Hannon

Howard Hughes Medical Institute (HHMI) Investigator and CSHL Professor Greg Hannon, Ph.D., was honored on April 15 with the 2011 Northeastern Association of Graduate School Geoffrey Marshall Mentoring Award. Greg is among the original faculty of CSHL's Watson School of Biological Sciences, which opened its doors in 1999. Since then, he has mentored some 12 postdoctoral fellows and 17 graduate students. The achievements of his trainees have been outstanding. Four students completed their Ph.D. degrees in less than 4 years and two were recipients of the prestigious Harold M. Weintraub Graduate Student Award, which is presented to the top graduate students in life sciences in the United States.

Every mentee in Greg's laboratory has had at least one publication in a high-impact journal, and more than 60% of Greg's 200-plus scientific publications have had graduate mentee coauthorship. The training that he provides has resulted in the placement of 10 of his graduate students in prestigious postdoctoral positions, with four already holding independent faculty positions at Mt. Sinai School of Medicine, the University of Toronto, the Fred Hutchinson Cancer Research Center, and the Whitehead Institute.

R. Martienssen

The HHMI and the Gordon and Betty Moore Foundation in 2011 selected CSHL Professor Rob Martienssen as one of the nation's 15 most innovative plant scientists to become an HHMI Investigator. The honorees will share $75 million in research funding over 5 years. Rob is a trailblazer in unraveling epigenetic mechanisms, which help to regulate how genes work, and is an expert on transposons—sequences of DNA that jump around the genome, often altering gene activity.

The Brain & Behavior Research Foundation (BBRF) awarded CSHL Professor Josh Huang a NARSAD Distinguished Investigator grant to study how genetic alterations associated with behavioral symptoms of schizophrenia disturb the development and function of neural circuits. Using a genetically engineered mouse strain, Josh will study chandelier cells, which are key to inhibitory circuits in the brain's frontal areas. NARSAD Distinguished Investigators are selected by BBRF's 124-member Scientific Council.

CSHL Fellow Chris Vakoc was selected by the Burroughs Wellcome Fund to receive a 2011 Career Award for Medical Scientists. The 5-year $700,000 award helps M.D.-Ph.D.s—a unique set of scientists who are experienced in both research and patient care—to bridge postdoctoral training and the early years of faculty service. This award supports Chris' work in identifying epigenetic vulnerabilities in chemotherapy-resistant leukemia.

Professor W. Richard McCombie was named a Fellow of the American Association for the Advancement of Science (AAAS) "for distinguished contributions in the areas of molecular biology,


J. Huang


C. Vakoc


W.R. McCombie

computational biology, and genomics, including high-throughput genome sequencing, and as director of the CSHL Genome Center."

Assistant Professor Hongwu Zheng was selected by the V Foundation for Cancer Research as a 2011 V Scholar. Additionally, his proposal to study the genetic and functional characterization of epidermal growth factor receptor (EGFR)-targeted therapy resistance in malignant gliomas received special attention as the winner of the Martin Abeloff award for outstanding project submission.

CSHL and the National Institutes of Health (NIH) cohosted the "NIH New Investigator Regional Conference" on March 14. The brainchild of the head of our Office of Sponsored Research, Walter Goldschmidts, and supported by The Alfred P. Sloan Foundation, the event, held at Grace Auditorium, brought key NIH directors and scientific program leaders together with more than 250 new faculty from 56 universities and research centers to discuss national biomedical research priorities and issues facing new investigators. Despite NIH programs designed to facilitate the transition of new scientists to independently funded principal investigators, the average age at which an investigator first obtains an initial independent research grant remains unacceptably high.

CSHL's National Cancer Institute (NCI)-designated Cancer Center successfully competed for and secured a renewal of its NCI grant that provides more than $4 million annually for research during the next 5 years. CSHL has been an NCI-designated Cancer Center since 1987.

Promotions

Alea Mills was promoted to Professor and two of her colleagues, Raffaella Sordella and Hiroyasu Furukawa, are now Associate Professors. Former CSH Fellow Christopher Vakoc was appointed Assistant Professor.


NIH New Investigator Regional Conference

Administrative leaders of CSHL were also recognized with promotions. Lari Russo was named Chief Financial Officer and Damian Desiderio is now the Comptroller. Hans Erik Aronson, who heads our Information Technology Department, was promoted to Chief Information Officer.

In the Development Department, Diane Fagiola assumed a new role as Senior Director, Philanthropy, and Karen Orzel was named Director of Annual Giving and Donor Relations.

Departures

HHMI Investigator Scott Lowe took on a new position at Memorial Sloan-Kettering Cancer Center, continuing collaboration with CSHL as an Adjunct Professor and active collaborator on a program project grant from the National Cancer Institute.

Education Programs

The Watson School of Biological Sciences

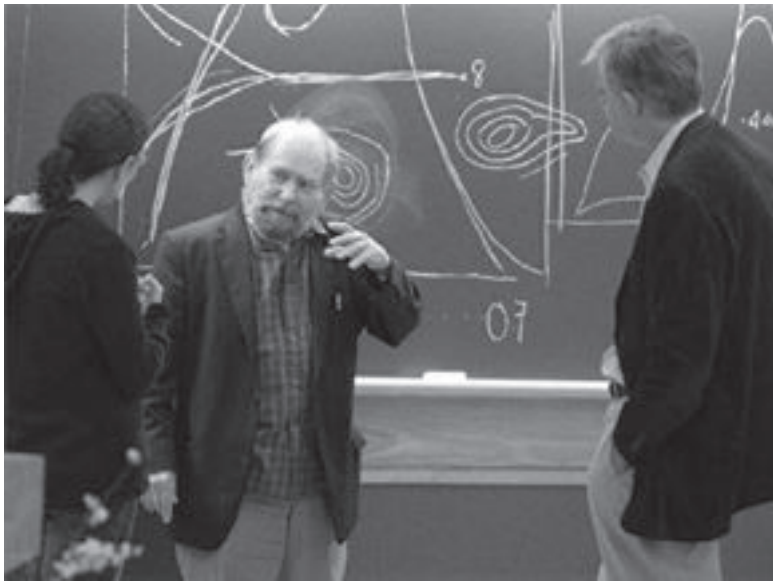
The School held its eighth graduation in 2011, with Amy R. Rappaport and Claudio Scuoppo joining the ranks of WSBS doctoral degree recipients, now numbering 49. Honorary degrees were bestowed on James H. Simons, Ph.D., Board Chair of Renaissance Technologies LLC, and James R. Lupski, M.D., Ph.D., Professor of Molecular and Human Genetics at Baylor College of Medicine.

Watson School graduates have thrived in the professional world. They publish in top scientific research journals and secure prestigious independent positions, fellowships, and awards. Eleven graduates to date have secured tenure-track faculty positions and are now receiving federal grants and publishing papers as independent researchers. At the end of 2011, WSBS students had published more than 185 research papers, a remarkable record since the school only started graduating students 7 years ago.

The 2011 entering class was composed of 10 accomplished students from the United States, Canada, the United Kingdom, France, Spain, and Switzerland. A significant number of additional students studying at CSHL are from Stony Brook University, via a program established between CSHL and Stony Brook more than 30 years ago.

The 10-week CSHL research program for undergraduate students—affectionately called "URP"—convenes in the summer and provides some of the finest college students in the nation a priceless opportunity to conduct sophisticated research at the side of a CSHL investigator. In 2011, URP's 53rd year, 727 college students competed for 28 slots, a continuing mark of the program's relevance and stellar reputation.

(*Left to right*) CSHL President B. Stillman, honorary degree recipients J.H. Simon and J.R. Lupski, and 2011 Watson School graduates A.R. Rappaport and C. Scuoppo



S. Brenner and B. Stillman at the Gavin Borden Lecture

The Gavin Borden Visiting Fellow Lecture "Reading the Human Genome" was presented on April 25 by Nobel laureate and long-time friend of CSHL Sydney Brenner, Ph.D., F.R.S.

Meetings and Courses Program

The world-renowned CSHL Meetings and Courses program attracted record attendance in 2011. More than 7750 people attended scientific meetings and more than 1300 (trainees, teaching, and support faculty) took part in courses. The Cold Spring Harbor Asia program based in Suzhou, China, meanwhile drew some 2700 participants to 13 conferences and a summer school held during 2011.

Our flagship meeting, the Cold Spring Harbor Symposium on Quantitative Biology, which each year focuses on a different topic, this year integrated classical biochemistry and modern molecular biology under the theme of *Metabolism and Disease*. This year's Symposium, the 76th in the historic annual series, featured 63 speakers from North America, Europe, and Asia. Steeped in the tradition of interactions between visiting scientists and the local community, the Dorcas Cummings Lecture opened the doors of the meeting to members of the local community, who were enthralled by Cyn-

Scene from the 76th CSHL Symposium

thia Kenyon's talk on "The Deadly Sweet Tooth." Dinner parties at local homes after the Lecture were the perfect venue to continue discussions of science and society.

New CSHL meetings in 2011 included *Stem Cell Engineering* and *Cell Therapeutics* and *The Biology of Cancer: Microenvironment, Metastasis, and Therapeutics*, both reflecting the increased interest in translational science. Basic bioscience meetings, such as those on *Eukaryotic Transcription* or *Neurobiology of* Drosophila, continued to flourish, whereas the *Eukaryotic RNA Processing* meeting was historic not least for beginning with an earthquake and ending with a hurricane.

CSHL offers ~25 high-level courses that attract more than 1000 scientists each year. Directed primarily at predoctoral-, postdoctoral-, and faculty-level scientists, these intensive courses allow practicing researchers to immerse themselves in new techniques and ideas that they can apply immediately to their own research. The HHMI awarded the Laboratory a new 4-year grant totaling $2.475 million to support current courses and to allow CSHL to start new courses.

HHMI

Banbury Center

In its 34th year of operation, the Banbury Center hosted 24 scientific meetings as well as six lecture courses, two Watson School courses, and a variety of third-party events in 2011.

The schedule centers around private scientific gatherings, and during the year, these were attended by ~686 participants from the United States and 25 other nations. Highlights of the science program included a series of meetings coorganized by James D. Watson. Dr. Watson and Joseph Schlessinger organized the meeting on *Curing Melanoma and Other Cancers by Targeted Therapies* that was well timed, coinciding with reports of exciting results in treatments with vemurafenib, developed by Plexxicon and Hoffmann La-Roche and now approved by the Food and Drug Administration. While the meeting was in session, the FDA approved ipilimumab for the treatment of metastatic cancer. Dr. James Allison, whose work helped lead to the development of the drug, was present at the meeting. Other timely cancer-themed meetings in the series included *Myc and the Pathway to Cancer* and *Metformin and Neoplasia*, both focusing on recent developments in discovering targeted therapies for cancer and dealing with cancer's unusual metabolism.

Outside groups use the Center for training young scientists, and we have developed close relationships in particular with the National Institute of Mental Health (NIMH) and the Boehringer Ingelheim Foundation. This year's NIMH "brain camp" was attended in full by the Institute's director, Dr. Thomas Insel. Banbury workshops in 2011 focused on areas of intense interest including autism spectrum disorders and pancreatic cancer.

DNA Learning Center

The DNA Learning Center (DNALC) continues to convey the wonders of molecular biology to young people, K–12 teachers, college faculty, and members of the general public. To date, the DNALC has introduced the concepts and technologies central to the field to more than 600,000 people. In 2011, 28,648 local students participated in labs, through field trips to DNALC facilities in Cold Spring Harbor, Lake Success, and Harlem, New York City, and via in-school instruction and the use of equipment footlockers.

More than 2800 of these students were from underserved Long Island and Queens school districts, and 60.2% of *Harlem DNA Lab* students in upper Manhattan were members of ethnic groups that are underrepresented in science. Enrollment in the DNALC summer camp programs set a new record, exceeding 1000, and collaborations with Cold Spring Harbor High School, the Watson School of Biological Sciences, and Stony Brook University continued.

Educator training was expanded in 2011: More than 1000 educators took part in training workshops across the United States, and more than 1600 people attended presentations or short workshops at professional meetings. In a continuing partnership with the New York City Department of Education, funded by the HHMI, the DNALC also provided training at the *Harlem DNA Lab* to 128 teachers in 44 1-day workshops, plus 2-week leadership training to 18 highly qualified teachers.

In late 2011, the DNALC portal (www.dnalc.org) was awarded the *Science* Prize for Online Resources in Education (SPORE). An essay in *Science* by DNALC founder and executive director David Micklos and two members of the team, Sue Lauter and Amy Nisselle, described the evolution and impact of the DNALC's suite of websites, apps, and YouTube resources that attract more than 7 million visitors annually. In 2011 alone, the DNALC added three more websites, a new "app," and an e-book to the portal.

In 2010–2011, studies were undertaken to measure the effects of DNALC programs on students. When compared with other educational methods and media, use of the two tested DNALC sites, *Inside Cancer* and *Genes to Cognition* (*G2C*) *Online*, helped increase student academic performance, on average, by one letter grade.

CSHL Press

The goal of the Press is to identify important research, technologies, and scientists; amplify their effectiveness by selecting, aggregating, and curating information research communities can use; and deliver that information when, where, and how the communities want it.

During 2011, research articles published by the Press' seven scientific journals continued to have a high impact in their respective fields; more than 2100 electronic journal subscriptions were fulfilled at academic institutions in 150 countries, including all leading American universities, and 39,600 copies of print books were shipped. This reach is complemented by interest from foreign publishers in translating certain books into languages such as Chinese, Japanese, Arabic, and most recently, Vietnamese.

As measured by impact factor, the best-known metric of worth, all CSHL journals advanced in 2011. *Genes & Development* and *Genome Research* continue to occupy the first tier of genetics, biotechnology, and developmental biology journals, and of the 8000 most important science journals published in the world, they are within the top 1.2%. All of the journals exceeded financial expectation, and *Cold Spring Harbor Protocols*, in its 5th year, had a remarkable increase in subscriptions. A new journal, *Cold Spring Harbor Perspectives in Medicine*, was launched on schedule in September, with papers on AIDS, Alzheimer's disease, and Parkinson's syndrome.

Seventeen new books were published during the year, bringing the number of available titles to more than 150. Former Trustee James Darnell's book, *RNA: Life's Indispensable Molecule*, published in July to critical acclaim, was named one of the 25 Outstanding Academic Titles for 2011 by *Choice* magazine. The best-selling titles of the year were the classic handbooks *At The Bench* and *At The Helm*, the iconic manual *Molecular Cloning*, and the magisterial history of molecular biology *The Eighth Day of Creation* by Horace Freeland Judson, whose passing at the age of 80 during the year we noted with great sadness.

The mission of the Press is being profoundly altered by changes in distribution channels. Laboratory protocols and topic-specific review articles are two types of content for which the Press has been renowned. Five years ago, that content was delivered only in printed books. Now, it comes also in the much more usable form of online-only, subscription-based review journals made possible by the reputation of those print books and the strategies used in creating them. Thus the same content types now have an additional means of delivery and a different, more robust business model, but the same exceptional quality. E-books and device apps are being explored. All Press journals online are now optimized for reading on mobile phones and tablet devices.

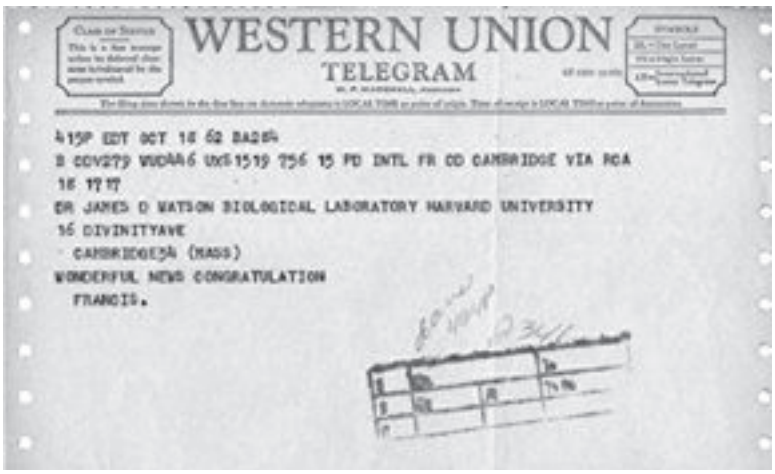
Library and Archives

Part of CSHL's broad education mission, the Library and Archives continued to promote open access to science history through grant-supported digitization projects.

Supported by wellcome trust

Backed with a generous $600,400 grant from the Wellcome Trust and with the assistance of photographer Ardon Bar-Hama, who has digitized the Dead Sea Scrolls at the Israel Museum and the archives of the New York Philharmonic, CSHL is now rendering nearly all of its comprehensive archives of Dr. Watson's papers, manuscripts, lectures, and many of his photographs in high-resolution digital format. Altogether, more than 250,000 Watson items will be digitized, as well as more than 100,000 items from the collection of Sydney Brenner. It is part of the Wellcome's 2-year pilot project on the theme of *Modern Genetics and its Foundations* that seeks to digitally "reunite" archival collections of the major players in the development of molecular biology (in addition to Watson and Brenner, these include Francis Crick, Maurice Wilkins, and Rosalind Franklin, among others). The collections will be made available through the Wellcome Digital Library website and our own CSHL Library's Archives Digital Collections Database.

Through a grant from the New York State Documentary Heritage Program Basic Processing Project, CSHL will arrange and describe three collections that represent CSHL's predecessor institutions, providing insights into the lives of scientists working at the Lab from 1890 to 1974. This will include



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TELEGRAM

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FRANCIS.

Item from the James D. Watson Archive

E. Watson, P.J. Kennedy, J.D. Watson, C. Moraetis, G. Echt, and S. Echt, at the President's Council meeting

material from the Eugenics Record Office, an organization that also existed in the Cold Spring Harbor neighborhood from 1910 to 1939.

Other notable initiatives undertaken by the Archives include a grant from the National Archives and Records Administration/National Historical Publications and Records Commission Basic Processing Project to process 630 linear feet of CSHL archival collections and a Preservation Assistance Grant for Smaller Institutions from the National Endowment for the Humanities that allowed for a general preservation assessment of our collections and a preservation workshop attended by institutional librarians and archivists across the New York metropolitan area.

Development

The Honorable Patrick J. Kennedy, cofounder of "One Mind for Research," opened the 27th annual President's Council on October 14, greeting donors whose $25,000 gifts support CSHL's most talented young scientists. The retreat focused on "The Science of Addiction" and featured Susan Foster, Columbia University; Joanna Fowler, Brookhaven National Laboratory; Philip Low, Chairman, Neuro Vigil; Eric Nestler, Friedman Brain Institute and Mount Sinai School of Medicine; and Howard Shaffer, Harvard Medical School. CSHL Assistant Professor Adam Kepecs discussed his work on neural circuit principles behind decision making, which he hopes will lead to better treatments for diseases such as addiction. We thank event chairpersons Howard Morgan, Cynthia R. Stebbins, and Steve Wiggins.

P.J. Kennedy, B. Stillman

Double Helix Medals Dinner

At our sixth Double Helix Medals dinner, $3.3 million was raised to strengthen and expand research and education programs.

The Double Helix Medal honors individuals who have positively impacted human health by raising awareness and funds for biomedical research. Each of this year's honorees—Temple Grandin, Harold Varmus, and Kareem Abdul-Jabbar—has made extraordinary contributions that have helped to transform the way in which doctors, patients, and society approach all types of cancer and neurological conditions such as autism.

Double Helix Medal Winners H. Varmus, K. Abdul-Jabbar, and T. Grandin

Infrastructure Projects

The Laboratory received a National Science Foundation Infrastructure Improvement Grant for greenhouse renovations at Uplands Farm. Growth facilities were renovated and improved, and all

Hooper House

three greenhouses received lighting, ventilation, and control system upgrades necessary to accommodate the evolving plant research program.

The circa 1830 Hooper House received a facelift in 2011. The entire exterior structure was renovated, with new cedar siding, windows, doors, and trim work. The existing wood and aluminum windows were replaced with modern equivalents that recall the 1830 originals. The renovation offered the Laboratory the opportunity to restore gable details and a central front porch that had been lost during earlier renovations.

Originally built as a single-family residence in the late 1960s, Osterhout was renovated to serve as the new home of the Laboratory's Public Affairs Department. The structure was gutted, reinforced, and modernized for current office needs, but its original exterior character was retained.

The Olney Barn, built in the late 19th century as a companion to the nearby Olney House, was in drastic need of renovation, but a survey of the structure and the site showed that it was impractical to renovate the existing structure. To provide a suitable workspace for the Laboratory's groundskeepers, we constructed a new barn at a more suitable location.

Concerned both with the health of its employees and with rising healthcare expenses, the Laboratory contracted North Shore–LIJ Health System (NSLIJ) to operate an on-site wellness center. The center was constructed in the space formerly occupied by two sleeping rooms in Dolan Hall. An NSLIJ Nurse Practitioner provides confidential health counseling and wellness services to CSHL employees and graduate students.

Osterhout Cottage

Olney Barn

Opening of the Center for Health and Wellness

Hershey Building

Great progress was made during the year on construction of the new, nearly 16,000-square-foot Alfred D. Hershey Building. Construction began in 2010 and is expected to be complete by the spring of 2012, just in time for summer courses. Funded by the HHMI, the building will also be home to the Laboratory's microscopy facilities, the Flow Cytometry shared resource, one course laboratory, and two spacious seminar rooms.

Our Hillside Laboratories, opened in 2009, continue to be recognized for their innovative architecture. In 2011, the project (designed by Centerbrook Architects) was recognized by the American Institute of Architects Connecticut Design Award and was a finalist in the *World Architecture News* Colour in Architecture competition.

Information Technology

CSHL is committed to staying ahead of the information technology curve and accordingly has made considerable investments during the last several years. Our plan has been to provide for strong IT disaster recovery/business continuity preparedness; a unified, redundant high-speed data network; and highly available, large-scale storage for scientific data.

In 2011, we successfully commissioned our Business Continuity/Disaster Recovery environment, which maximizes availability of core IT resources through mirroring of data and services and providing instant, nondisruptive restoration of mission-critical resources. The CSHL campus high-speed fiber-optic data network was upgraded to provide access to high-bandwidth applications at additional CSHL facility locations, including the Banbury Conference Center, Uplands Farm, and the Knight House.

The entire campus now also enjoys fully redundant, dual-path connection to our Internet service provider (ISP) and a 10-fold increase in bandwidth product, from 100 Mbp to 1 Gbp. Our research is better protected as a result of an improved data backup system and the expansion of data storage systems for scientific computing (high-speed sequencing and data analysis). CSHL's IT department currently manages more than 3 PBs of research data!

Community Outreach

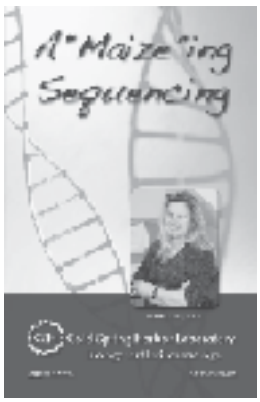
CSHL's guided Walking Tour Program was enjoyed by more than 600 visitors in 2011, who came from nearby communities as well as faraway lands including Singapore, Japan, Korea, Brazil, and Germany. We thank our team of a dozen graduate students and postdocs who are the docents.

Local families battled the inclement April weather to explore the history of Cold Spring Harbor Village's Main Street and DNA on April 16–17 as part of the Lab-sponsored DNA Day Scavenger

DNA Day Scavenger Hunt map

Hunt. Neighboring educational organizations such as the Cold Spring Harbor Public Library and Environmental Center, the Cold Spring Harbor Whaling Museum, the Firehouse Museum, and our own DNA Learning Center all took part.

DNA Day is celebrated across the country, with educational events sponsored by the National Human Genome Research Institute (NHGRI), a part of the National Institutes of Health.



Orlando Science Center poster announcing Doreen Ware's talk

In Orlando, Florida, visitors to the Orlando Science Center's DNA Day celebration engaged in Skype video conference sessions with CSHL faculty members Dick McCombie and Doreen Ware and Research Investigator Emily Hodges. Among the three scientists, they covered DNA-relevant topics ranging from genome sequencing and plant biology to Neanderthal bones.

Seventy-five winners from the 2011 Brentwood District Science Fair celebrated DNA Day by genetically engineering bacteria that glow. Funded by the National Grid Foundation, the hands-on scientific experiences delivered by the DNALC are just one example of the ways in which the DNALC partners with school districts to enhance biology curricula.

At the fourth annual World Science Festival in Manhattan, the DNALC showcased the Urban Barcode Project as part of the festival's lively Street Fair event. The city-wide barcode project competition, funded by a grant from the Alfred P. Sloan Foundation, has attracted 103 high school teams and will culminate in June 2012 with the award of scholarship prizes to students on winning teams.

NYC URBAN BARCODE PROJECT

CSHL continued its partnership with the Long Island 2-Day Walk to Fight Breast Cancer, judging the annual scholarship essay contest and cheering on walkers during the event. Our faculty, staff, and students participated in many other fundraising events that benefit our research; they are organized by disease-based organizations in our local community including Swim Across America, the Don Monti Memorial Research Foundation, the Joni Gladowsky Breast Cancer Research Foundation, and the Manhasset Women's Coalition.

The year 2011 became the year that CSHL broke into the social-media world, launching an institutional Facebook page, blogging on our own LabDish page, and amassing a significant Twitter following. Our monthly electronic *NetLetter* won national acclaim from PR News for its highly interactive and visually appealing platform, which delivers news and information about CSHL that is both useful and relevant to a diverse and growing audience.

Public Lectures at Cold Spring Harbor Laboratory


P. Glimcher

April 4—Paul W. Glimcher, Ph.D., Julius Silver Professor of Neural Science, Economics, and Psychology and Director for the Center for Neuroeconomics, New York University: *Neuroeconomics and the Biological Basis of Decision-Making*.

June 22—Fritz Henn, M.D., Ph.D., CSHL Professor; **Husseini Manji, M.D.**, Global Head of Neuroscience at Johnson & Johnson Pharmaceutical Research & Development, LLC; *New Approaches to Treatment of Depression and Bipolar Disorder*, cosponsored by CSHL, Brain & Behavior Research Foundation, Bank of America-Merrill Lynch, and St. Johnland Nursing Center.

July 6—Partha Mitra, Ph.D., CSHL Professor: *Mapping the Mouse Brain*, hosted by the Secret Science Club, Brooklyn, New York.

August 24—Rob Martienssen, Ph.D., CSHL Professor and HHMI Investigator: *Send in the Clones: Superseeds, Superweeds, and Green Energy*, hosted by the Secret Science Club, Brooklyn, New York.

October 23—Anne Churchland, Ph.D., CSHL Assistant Professor; **Concetta M. Tomaino, D.A., M.T.-B.C., L.C.A.T.,** Executive Director/Cofounder, Institute for Music and Neurologic Function; Senior Vice President, Music Therapy Services, Beth Abraham Family of Health Services; **Irene Gubrud, D.F.A.**; Soprano, Voice, and Meditation Teacher; **Steven Finch, M.M.**; Conductor, Choral Clinician: *Music: How It Can Rewire Your Brain*, cosponsored by CSHL and St. Johnland Nursing Center.

CSHL Public Concerts

April 15:	Two Plus One, trio
April 29:	Jennifer Johnson, soprano
May 6:	Hiroko Sasaki, pianist
May 20:	Xun Wang, pianist
August 19:	Margarita Shevchenko and Lev Polyakin, pianist and violinist
September 9:	Bella Hristova, violinist
September 23:	Natalia Lavrova, pianist
October 14:	Yoonie Han, pianist

Looking Forward

As we look ahead, I remain confident that CSHL is well-positioned to fulfill its research and education mission, continuing to lead the field of molecular biology and genetics to its next major achievements. My confidence is based on this institution's faculty, staff, and students, who make my job as President such a source of pride. I thank you, our Board of Trustees, and generous donors for your support.

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

CHIEF OPERATING OFFICER'S REPORT

W. Dillaway Ayres, Jr.

Had one been told in advance that 2011 would be marked by an extraordinary natural disaster in Japan, unprecedented political upheaval in northern Africa, a European debt crisis, stubbornly slow economic growth and high unemployment in the United States, and a downgrade of the U.S. credit rating, one might have predicted a terrible year for endowment investment returns and an unusually difficult year for nonprofit institutions that depend on government funding and philanthropy. Fortunately, this was not the case at Cold Spring Harbor Laboratory.

The exceptionally high quality of the Laboratory's research and education programs allows us to weather challenging economic times more easily than many institutions. We have a comparatively high federal grant success rate, and we enjoy very healthy support from private foundations and individuals. The Laboratory has also established a unique portfolio of assets including an internationally renowned scientific Meetings and Courses program and a prolific, well-respected scientific publishing arm. These activities serve to diversify the institution from both an economic and intellectual perspective, as do our graduate school and DNA Learning Center programs.

Although spending from the endowment fund represents a relatively small percentage (11%) of the annual operating budget, endowment is nevertheless critical to the Laboratory's stability and future. The Investment Committee of our Board of Trustees works hard to ensure that the fund is well-diversified and positioned appropriately for growth and capital preservation.

In preparing the 2011 budget during the fourth quarter of 2010, we were mindful of two consecutive years of strong investment returns in 2009 and 2010, and we anticipated volatility in 2011. Consequently, we forecasted zero percent return for 2011, which is exactly where we finished. While not a particularly exciting result, we were pleased that the investment strategy successfully preserved capital in an extraordinarily volatile year. Looking forward, the Board of Trustees has targeted increasing the Laboratory's endowment fund as its highest priority in this period of flat federal funding for research.

For the fiscal year ending 12/31/11, the Laboratory's operating budget reached approximately $154 million, an increase of 5% over the prior year. Although top-line growth is good, it is important to remember that a portion of federal grant revenue still reflects funding made available through the American Recovery and Reinvestment Act, presumably a nonrecurring phenomenon. With Washington facing large deficits and election-year politics, we remain concerned about the levels of federal funding for research. Management's focus on driving revenue and controlling expenses led to a satisfactory operating result in 2011. However, in assimilating our recently expanded infrastructure and new faculty hires, we are drawing, as planned, on the "research start-up" fund raised in our last fund-raising campaign. This fund is scheduled to be fully spent by 2016–2017; hence, the emphasis on raising more endowment.

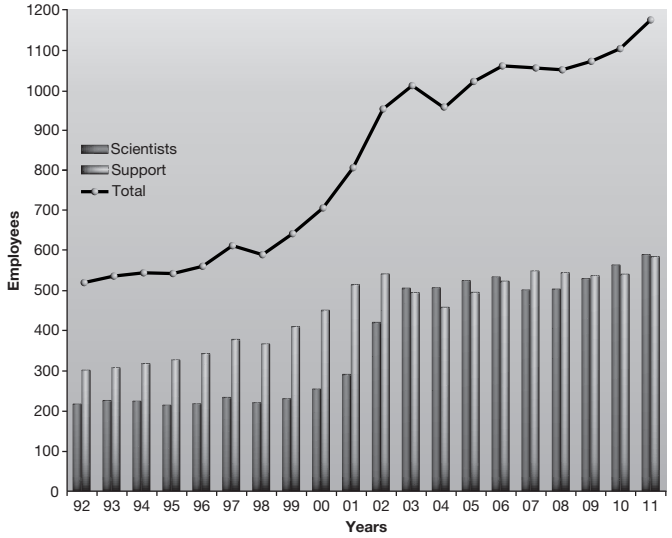
Complacency is hardly an option. The costs of doing innovative science continue to rise. Funding is uncertain. Remaining in the forefront of the field requires investment in equipment, infrastructure, and personnel. It is critical that we remain diligent about controlling expenses while preserving and building the resources required to address the world's most important health problems.

As always, we are grateful to all who support the Laboratory's work, and we are inspired by our talented team of scientists and staff, of whom we ask so much.

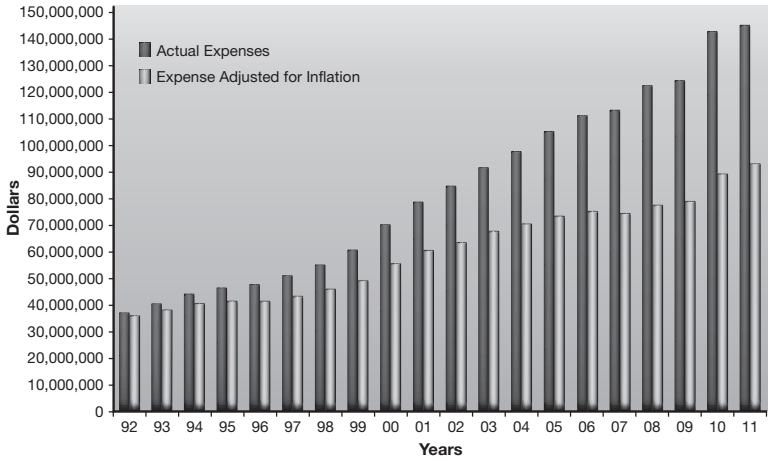
Dill Ayres
Chief Operating Officer

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)

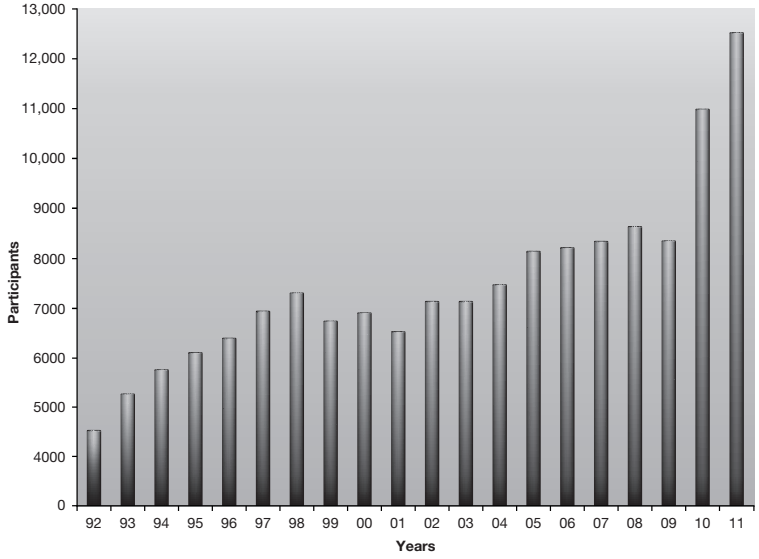


Operating Expense



Meetings and Courses Participants

(2010–2011 amount includes CSH Asia and Banbury Center meetings.)



Long-Term Service

Front row (*left to right*) Susan Lauter, Delia King, Patricia McAdams, Marlene Rubino, Patricia Wendel, Mary Ann Miceli, Barbara Zane; *top row* (*left to right*) Michael Regulski, Peter Stahl, Philip Renna, John Maroney, Bruce Stillman, Timothy Mulligan, Christopher Hubert, William Keen, Peter Schwind, Frank Russo

The following employees celebrated milestone anniversaries in 2011:

40 Years	Bill Keen, John Maroney
35 Years	Peter Stahl, Pat Wendel
30 Years	Rodney Chisum, Philip Renna, Marlene Rubino
25 Years	Chris Hubert, Adrian Krainer, Susan Lauter, Vincent Meschan, Tim Mulligan
20 Years	Kathy Cirone, Delia King, Mary Ann Miceli, Patricia McAdams, Chris Oravitz, Michael Regulski, Frank Russo, Peter Schwind, Judi Smith, Linda Van Aelst, Barbara Zane

Long-Term Service: 15 Years

Front row (*left to right*) Liz Powers, Marcie Siconolfi, Peggy Brock. *Top row* (*left to right*) Florecita Chaves, Kaaren Janssen, Bruce Stillman

15 Years	Scott Bennett, Peggy Brock, William Carmona, Florecita Chaves, Wendy Crowley, Constance Hallaran, Kaaren Janssen, Melissa Kramer, Oscar Lastra, Lorraine McInerny, Maureen Morrow, Liz Powers, Marcie Siconolfi

RESEARCH

See previous page for photos of the following scientific staff:

Row 1: O. Anczukow-Camarda (Krainer lab); S. Xu (Zhong lab); M. Cohen (Egeblad lab); M. Matlashov, V. Belousov (Enikolopov lab)

Row 2: N. Chatterjee (Dubnau lab); M. Ramesh (Tonks lab); D. Wah (Joshua-Tor lab); C. Kuscu (Joshua-Tor lab)

Row 3: K. Jiang (Lippman lab); A. Jespersen (Furukawa lab); E. Lee (Hannon lab); M. Penzo (Li lab)

Row 4: C. Carlston (Hammel lab); N. Azamy (McCombie lab); M. Javelle (Timmermans lab); H. Taniguchi (Huang lab); B. Zhang (Spector lab)

Row 5: T. Hige (Turner lab); B. Xue (Muthuswamy lab); R. Campbell (Turner lab); M. Vigliotti (Lucito lab)

CANCER: GENE REGULATION AND CELL PROLIFERATION

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

Christopher Hammell's lab is interested in understanding gene regulatory processes that give rise to robust phenotypes associated with normal development in animals (specifically, how the timing of developmental processes is controlled) as well as alterations in these pathways that give rise to diseases such as cancer (as in the alterations in mitogenic pathways in melanoma). To address this elemental problem, Hammell and colleagues are using a variety of model organisms and patient-derived cancer cell lines. To directly identify the components that function in controlling normal developmental timing, they are using forward and reverse genetic approaches with the small nematode *Caenorhabditis elegans*. In contrast to the extreme robustness of cell-fate lineage in *C. elegans*, in which specification of developmental programs is hard-wired, mutations that alter conserved signaling pathways in melanoma create relatively plastic developmental landscapes that allow these lesions to become aggressive tumors. Notably, the gene regulatory architecture of melanoma cells allows them to acquire resistance to therapeutic agents. Hammell's team is interested in epigenetic mechanisms that contribute to resistance, specifically, dramatic changes in gene expression patterns and intracellular signaling pathways. High-throughput screens are being performed in the Hammell lab to identify cellular factors that allow these rewiring events to occur, with the idea that these components would make ideal therapeutic targets to complement existing clinical strategies.

Leemor Joshua-Tor's lab studies 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. They use X-ray crystallography to obtain three-dimensional (3D) structures of individual proteins. Biochemistry and molecular biology enable them to study properties that can be correlated with protein structure and function. It was Joshua-Tor and her team who first obtained the structure of a full-length Argonaute protein, work that instantly solved a long-standing puzzle in the RNA interference (RNAi) field. By observing the structure, they realized that Argonaute was the long-sought Slicer, which performs the critical slicing event in RNAi, cleavage of messenger RNA. Joshua-Tor is also well known for her work on the E1 helicase enzyme, which acts to unwind DNA strands during the DNA replication process. This past year, Joshua-Tor and colleagues determined the 3D structure of a protein complex called RNA-induced initiation of transcriptional gene silencing (RITS), discovering new details of how its various domains contribute to heterochromatin assembly and gene silencing. Joshua-Tor and colleagues also solved the crystal structure of the complex of Gal3p–Gal80p in the yeast *Saccharomyces cerevisiae*, with α-D-galactose and ATP—a classic transcription complex with its two ligands. This work revealed that the Gal3p transducer of the *GAL* regulon interacts with the Gal80p repressor in its ligand-induced closed conformation.

Adrian Krainer's lab studies mechanisms of RNA splicing, ways in which they go awry in disease, and 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 an mRNA splicing defect in SMA that makes a gene called *SMN2* only partially functional forms the basis of a potentially powerful therapeutic approach. In recent years, the team identified a compound that stimulates SMN protein production by altering RNA splicing. By introducing chemically modified pieces of RNA called antisense oligonucleotides (ASOs) into the spinal cords of mice, they succeeded in reversing symptoms of Type III SMA. This year, the team was surprised to discover that systemic—as opposed to central nervous system—injection of ASOs

in mice with severe SMA a day after birth increased their survival 10-fold to 25-fold, depending on dose. This suggests that correcting *SMN2* splicing in tissue other than brain and spinal cord could significantly improve outcome. The lab's expertise in splicing has also helped to shed light on the abnormal glucose metabolism of cancer cells, sometimes referred to as the Warburg Effect.

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 the examination in real time of the recruitment of members of gene expression and silencing machineries. One of their current research focuses is on understanding nuclear positioning of genes in relation to their transcriptional activity. Another focus is on the identification and characterization of long nuclear retained noncoding RNAs (ncRNAs), whose functions are still poorly understood. Using 4D live cell imaging during the past year, the team discovered that the formation of a type of nuclear subcompartment called the paraspeckle is triggered by a pair of ncRNA molecules, which also maintain its structural integrity. In additional work published this year, Spector's team measured the kinetics of activation of a single gene locus—the specific chromosomal location of a gene—in a cell before it divided and compared it with the same gene's reactivation in newly formed daughter cells. In this way, they determined how "bookmarking" a gene predivision marks it for reactivation postdivision. The bookmark is an acetylated histone that recruits the protein BRD4 to the gene locus during mitosis. Their findings revealed a function of BRD4 in chromatin decompaction that allowed for the rapid kinetics of gene activation in postdivision cells.

Arne Stenlund and colleagues have obtained a detailed understanding of processes required for initiation of DNA replication from papillomaviruses, using this system to gain a general biochemical understanding applicable to other systems. Papillomaviruses are a large viral family that induces cell proliferation at the site of infection, usually giving 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. 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 the cells divide in two. Working with yeast and human cells, Stillman and colleagues have identified many of the cellular proteins that function at the DNA replication fork during the S phase, the portion of the cell-division cycle at which DNA synthesis occurs. Among these proteins are those that facilitate the assembly of chromatin, the protein–DNA complexes that form the chromosomes. The primary focus of current research, however, is on 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 the ORC is also involved in the process of segregating duplicated chromosomes in mitosis. The team has found ORC at centrosomes and centromeres, structures that orchestrate chromosome separation in mitosis. This year, Stillman's team took part in research that demonstrated the feasibility of using RNAi technology to switch genes on and off in a nonlethal manner, a boon to disease research in animal models. They "turned off" a gene necessary for DNA replication in mice until the animals became moribund and then successfully reversed this condition and rescued the mice by turning this gene back on.

MODULATION OF microRNA ACTIVITY IN DEVELOPMENT AND DISEASE

C.M. Hammell C. Aquirre-Chen A. Levin
C. Carlston A. Turberfield
D.M. King

The temporal regulation of gene expression is an indispensable characteristic of metazoan development and is the basis for the progressive control of genetic programs that coordinate animal growth and aging. *Caenorhabditis elegans* is an ideal model organism whose invariant, well-characterized cell lineage can be leveraged to determine the mechanisms that couple these processes. Genes that control the patterning of temporal cell fates, including microRNAs (miRNAs) and their targets, are expressed at defined larval stages and are temporally down- or up-regulated accordingly. How these regulatory processes are controlled and coordinated during animal development is a major focus of our laboratory.

Genetic Approaches to Identify Components That Modulate the Temporal Activity of miRNAs

C. elegans, as a model organism, has provided an unparalleled system in which the principles of temporal cell-fate specification can be examined. Characterization of mutants that alter the patterning of cell-fate determination has revealed two central precepts of developmental timing that are conserved throughout evolution. The most fundamental maxim to emerge from these studies is that the genes which control the patterning of cell fate operate within defined modular regulatory programs and each program is limited to a single stage of development. Importantly, the activity and sequential expression of these individual modules is integrated into a much larger, linear gene regulatory network. This larger regulatory network is punctuated by four molting periods in which the hypodermal cells synthesize and secrete a new cuticle and separate from one produced in the prior stage. The coordination of the molting cycles, the individual cell divisions, and the regulation of temporal cell fate specification are precisely timed, allowing the events of cell specification to occur in absolute developmental time (as measured from hatching) or relative to reiterated patterns of cuticle formation expressed during the molting periods. Heterochronic mutants can be classified into two groups that reflect their distinct effects on the patterning of temporal cell-fate specification. Precocious mutants execute later stage-specific cell division programs at earlier times by skipping intermediate stages of development and display adult-specific gene expression programs during larval development. Conversely, retarded heterochronic mutants reiterate individual cell-fate programs and inappropriately retain larval features in adulthood.

The modular nature of temporal cell-fate specification is also underscored by a regulatory strategy that is used multiple times during development. This strategy uses variations on the theme of sharp transcriptional induction of specific miRNAs and the rapid reduction of their target transcripts. This serial and progressive regulation of heterochronic genes limits the activity of individual cell-fate specification programs to a single larval stage. Animals that lack discrete heterochronic miRNAs continuously reiterate early cell lineage specification programs as a consequence of continued, inappropriate expression to the mRNAs normally targeted by each miRNA (Fig. 1). These mutations result in specific alterations of multiple cell lineages during postembryonic aging and are easily distinguished from wild-type animals via multiple observable criteria (including stage-specific reporters, total number of cells, and the presence or absence of adult structures). The mechanisms and components that ensure the temporal precision of miRNA-mediated expression (and therefore couple development processes with chronological aging) are unknown.

Our approach to this problem uses a genetic pipeline that filters multiple candidates through two powerful genetic screens that are conducted in series. This approach has enabled us to isolate gene products that modulate the expression of specific miRNAs (see below). From candidates identified in primary screens, components that coordinate general miRNA-mediated processes can be identified in secondary screens. To identify these regulatory candidates, we intentionally focused on finding suppressors of specific miRNA mutants that produce mature 21-nucleotide regulatory

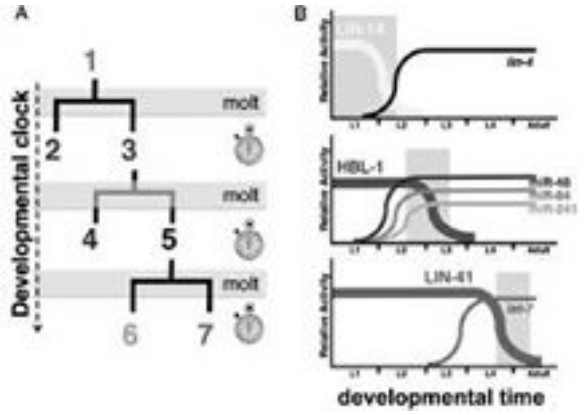


Figure 1. *C. elegans* development proceeds through a strict series of specific cell-fate patterns that are punctuated by molts. (*A*) At each larval molt, many somatic cells divide and display distinct cell fates and gene expression patterns. In normal animals, temporal patterns of cell specification are "hard-wired" and control heterochronic genes that directly modulate specific developmental programs as well as genes that demarcate relative developmental time. (*B*) The patterning of developmental cell-fate specification is controlled by several miRNAs that accumulate at distinct stages of development and down-regulate the expression of other gene products that control larger suites of temporally regulated genes.

RNA products yet fail to efficiently down-regulate their target transcripts. Animals harboring *lin-4(ma161)* or *let-7(n2853)* mutations (which alter a single nucleotide in the mature "seed sequence" of the miRNA) display specific retarded cell lineage phenotypes at early and late stages of development and underaccumulate functional *lin-4* and *let-7* miRNAs, respectively. Phenotypes associated with these alleles are indistinguishable from those displayed in animals harboring null mutations of these genes. To identify components that control miRNA-dependent processes during the middle stages of larval development, we also used a novel antimorphic allele of the *C. elegans* miRNA-specific argonaute (*alg-1*(*ma192*)) that exhibits retarded heterochronic phenotypes due to the inappropriate perdurance of a single miRNA target.

Primary screen. At the cellular and molecular levels, these mutations result in animals that display distinct cell lineage phenotypes and disrupt defined genetic interactions. Each of these phenotypes is caused by a general inability of animals to efficiently down-regulate specific miRNA targets, which ultimately prevents the progression of the normal regulatory cascade that culminates in adult-specific gene expression. These mutual macroscopic defects are easily monitored through the use of a reporter (*col-19pro::GFP*) that is only expressed during adulthood in wild-type animals. Candidate suppressors of these phenotypes are identified as genes that, when mutated or depleted via RNA interference (RNAi), recapitulate the normal temporal expression pattern of *col-19::GFP* in mutant animals (primary screens; Fig. 2). This screening step, by itself, is analogous to previous methods used to isolate heterochronic genes, which have identified many important mutants, and yet remain unsaturated.

Secondary screen. Our strategy uses the power of these unbiased approaches but is distinguished from prior screens by the application of a refinement procedure that isolates, from the suppressors identified in primary screens, those gene products that normally function to promote the precise expression of multiple temporally regulated miRNAs. Specifically, suppressors identified in the primary screens are queried for their ability to correct additional heterochronic phenotypes associated with particular developmental programs occurring at different larval stages. This secondary screening process is both powerful and direct, as the retarded developmental phenotypes displayed by each of these genetic contexts result from a common inability to express an unambiguous reporter of adult-specific expression (*col-19::GFP*). From the information gained in our pilot screens, this secondary genetic application appears to directly enrich for regulatory components that control miRNA expression within the context of the entire regulatory cascade controlling the patterning of postembryonic cell fates.

Identification and Characterization of Components that Couple Developmental and Chronological Ages

In the past year, we have completed pilot, EMS-based primary screens for each of the genetic backgrounds outlined above. Our efforts have resulted in the identification of 28 novel mutations that suppress at least one of

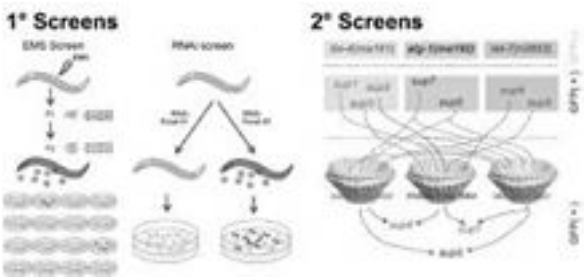


Figure 2. A two-part genetic strategy to identify genes that modulate the expression or activity of heterochronic miRNAs.

these genetic contexts. To focus on regulatory components of temporal expression, we are currently categorizing the resulting mutations from the primary screen for their ability to suppress phenotypes associated with the other hypomorphic miRNA backgrounds (secondary screens, Fig. 2). These alleles will be characterized using standard genetic techniques and whole-genome sequencing to assign complementation groups and identify the causative genetic lesions of these alleles, respectively.

We have already determined the capacity of several of these mutations to suppress the retarded heterochronic phenotypes of all three genetic contexts (*lin-4, let-7,* and *alg-1*), therefore establishing the simplicity and utility of this two-tiered screening strategy. Surprisingly, five alleles from the pilot EMS-based screens contain genetic lesions that lie within a single gene, *lin-42*, encoding the *C. elegans* homolog of the human and *Drosophila* Period genes implicated in circadian timing. Importantly, novel *lin-42* alleles were identified as suppressors in each of the above mutant backgrounds, testifying to the power of this genetic strategy. Although *lin-42* function has been implicated in controlling animal behavior, the molting cycle, and aspects of postembryonic development, we believe that the identification of *lin-42* mutations with this two-tiered approach allows us to directly interpret its function in the context of normal temporal gene regulation. These observations, for several reasons, suggest that *lin-42* activity is essential for the normal temporal coordination of cell-fate specification events that occur during larval development. First, a reduction in *lin-42* function suppresses the early, middle, and late cell lineage defects of distinct miRNA mutants, indicating that *lin-42* activity is required throughout larval development. This interpretation is also supported by the observation that *lin-42* encodes multiple nuclear proteins that increase in abundance before being cleared from somatic cells once per larval stage. This recursive, pulsatile protein accumulation is reminiscent of the periodic expression profiles of the human and *Drosophila* Period proteins controlling circadian gene expression. Genetic evidence also suggests that much of *lin-42* activity is mediated by and is dependent on the ability of animals to express mature *lin-4* and *let-7* miRNAs. Northern analysis of miRNA expression patterns in *lin-42* mutants indicates that the loss of *lin-42* activity results in the precocious and overabundant accumulation of specific heterochronic miRNAs (Fig. 3). Transcriptional green fluorescent protein (GFP) reporters, driven by the *lin-4* and *let-7* promoter sequences, indicate that *lin-42*-mediated regulation is controlled primarily at the transcriptional level. Furthermore, the

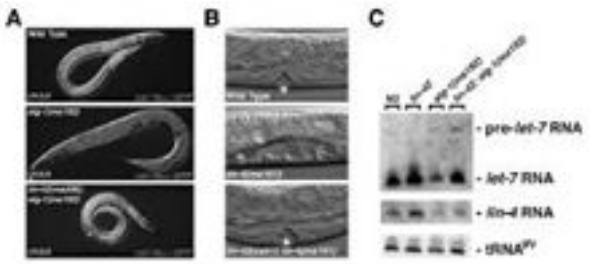


Figure 3. *lin-42*(*lf*) suppresses heterochronic phenotypes of developmental timing mutants that are defective in miRNA function. (*A*) In wild-type animals, the adult-specific expression of a *col-19::GFP* reporter is only expressed after the larval stages. In *alg-1*(*ma192*) animals, which harbor mutations in the *C. elegans* miRNA-specific argonaute protein, the proper expression of *col-19::GFP* is defective because the inability of these animals to down-regulate larval developmental genes is compromised. Removal of *lin-42* function in *alg-1*(*ma192*) mutants restores the normal down-regulation of larval gene expression. (*B*) *lin-4* (*ma161*) mutants reiterate early larval developmental cell fates and lack vulval structures. Removal of *lin-42* function in *lin-4*(*ma161*) animals corrects these temporal cell-fate defects. (*C*) Mutations in *lin-42* affect miRNA accumulation.

mild precocious heterochronic phenotypes of *lin-42(lf)* animals are dependent on the expression of specific miRNAs and are suppressed by reducing their overall levels. Consistent with this genetic dependency and its mechanistic role in controlling miRNA transcription, *lin-42* mutations completely fail to suppress retarded cell lineage defects associated with null alleles of *lin-4* or *let-7*, but they completely suppress hypomorphic alleles of multiple miRNAs. These results suggest that *lin-42* normally functions to reduce the steady-state accumulation of heterochronic miRNAs in a pulsatile fashion.

We have also screened ~25% of a genomic RNAi library in the *lin-4, let-7,* and *alg-1* mutant backgrounds. These efforts have identified at least two novel heterochronic genes: *pqn-59,* encoding a "prion-like" protein, and *E02D9.1b*, the *C. elegans* homolog of human Sam68, a conserved RNA-binding protein implicated in alternative splicing. Mutations in these genes recapitulate the RNAi-based suppression phenotypes observed in all three retarded miRNA mutant backgrounds, and RNAi depletion of *E02D9.1b* or mutations of *pqn-59, pqn-59(tm2960),* in an otherwise wild-type genetic background, results in the precocious expression of adult-specific genes.

Characterization of each suppressor identified by this strategy is being carried out at both the molecular and genetic levels. As each of the cell lineage defects in *lin-4, let-7,* and *alg-1* mutants are quantifiable, the suppression of each of these phenotypes (including the correction of

Figure 4. The new heterochronic genes *pqn-59* and *E02D9.1b* are ubiquitously expressed in *C. elegans* larva.

gene expression patterns, presence or absence of adult-specific cuticle structures, and changes in cell lineage) by each gene is also being characterized in detail. In addition, for genes identified through RNAi screening, we are constructing null and loss-of-function alleles using standard techniques. These reagents will be used to properly characterize phenotypes associated with complete loss-of-function alleles and order gene function (epistasis) with regard to the regulation of cell-fate specification genes and developmental oscillatory components (see below). In addition, transcriptional and translational reporters of these genes have been constructed and transformed into wild-type animals to ascertain the temporal and spatial expression patterns of these gene products (Fig. 4) (see GFP reporters for PQN-59 and E02D9.1b). These reporters will complement efforts using antisera directed against these proteins that will be used to identify the additional protein components that physically associate with these proteins in vivo via immunoprecipitation. In addition, for components that bind nucleic acids (such as E02D9.1b), these reagents can be used to identify RNA and DNA elements normally bound by these components in vivo.

Pervasive Levels of Temporal Gene Expression throughout *C. elegans* Development

In an effort to establish the depth and general dynamics of transcription that occurs during development and aging, mRNA expression profiles were obtained from wild-type nematodes at multiple stages throughout postembryonic growth. The temporal precision of these experiments revealed that, in addition to the transcriptional changes associated with the genes controlling temporal cell-fate specification, an astonishingly large portion of the *C. elegans* transcriptome is temporally regulated. About 40% of mRNAs exhibit pulsatile expression patterns and ~2800 mRNAs cycle in abundance during each larval stage. These findings compelled us to pose elemental questions about how developmental timing networks function in a progressive, unidirectional manner and how these processes are accomplished with such precision in the context of dramatic genome-wide cycles of gene expression. The pervasiveness and the periodicity of transcriptional cycling suggest that this oscillatory behavior is a fundamental biological property and supports the robustness of cell-fate specification.

Although *lin-42, pqn-59,* and *E02D9.1b* each encode unique classes of gene products (a nuclear protein homologous to components that regulate circadian gene expression, a "prion-like" protein, and an RNA-binding protein), each gene is periodically expressed at the protein and transcriptional levels. Importantly, these genes cycle in abundance from essentially undetectable levels to intense expression in a pulsatile fashion during each molting period. This recursive arrangement of expression is distinct from the essentially binary regulation displayed by other developmental timing genes. For instance, previously characterized heterochronic components, such as the miRNA targets *lin-14, hbl-1,* and *lin-41*, function as genetic "switches" controlling the intricate gene expression programs specific to a single larval transition (see Fig. 1). These genes accomplish this task by being expressed (switched on) at one or more developmental stages and then switched off. This sequential reduction of "switch-like" heterochronic gene expression facilitates the progressive elaboration of later cell fates by limiting the function of genes that have been utilized in earlier developmental programs. In contrast, new heterochronic genes such as *lin-42, pqn-59,* and *E02D9.1b* likely function as molecular clock components that demarcate both absolute and relative developmental time. Through their periodic expression patterns and ability to directly regulate expression of other "switch-like" heterochronic genes (such as miRNAs), these components integrate temporal cell-fate specification with animal growth. Importantly, these clock proteins cycle out of phase with the larval molts and retain this periodic behavior in the context of mutant animals that display alterations in the patterning of temporal cell fates. While experiments directed to understanding the molecular activities of each of these genes

are being pursued in a long-term and independent manner, the hypothesis that these genes coordinate the temporal expression of miRNAs and/or mRNAs is also being directly tested.

Preliminary analyses of temporal expression programs generated from RNA-Seq assays revealed an astounding level of phased gene expression throughout the life span of the worm. At a superficial level, these transcription phases appear to be directly coupled to the temporally controlled cell divisions and molting periods of postembryonic development (Fig. 5). Although one may suggest that these expression patterns represent a simple repeated cascade of derivative transcriptional processes that are initiated at the beginning of larval development, a detailed analysis of these expression patterns suggests that there are two distinct waves of transcriptional activation that occur during each larval stage. The first of these waves is initiated in the middle of each larval period, whereas the second wave occurs just before the molt, during cuticle formation and ecdy-sis, as shown for two examplar genes (Fig. 5). Peak *lin-42* expression is also pulsatile and remarkably occurs in between these two developmental peaks. This suggests that developmental clock components, such as *lin-42, pqn-59,* and *E02D9.1b,* may function to delineate this phasing process.

To directly assay the roles these clock components have in regulating both mRNA and miRNA temporal expression patterns, we have developed a dual reporter system that enables us to simultaneously measure the transcription of a variety of genes in living animals. The basis of this strategy has been used to measure the stochasticity of gene expression in single-celled organisms

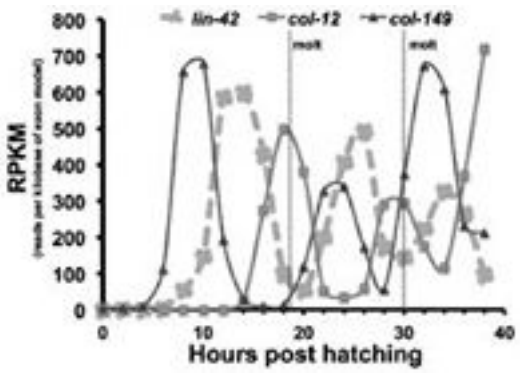


Figure 5. *C. elegans* transcription is pervasively regulated at the temporal level, and many genes display transcriptional profiles that are anti-phasic to the expression of *lin-42*. *col-149, lin-42,* and *col-12* transcripts are expressed once each larval stage. Importantly, the phasing of transcripts relative to each other is maintained for each gene every larval period.

and is both extremely sensitive and quantitative. This strategy involves simultaneously monitoring two genes in each animal, tagged with either GFP or mCherry. Both of these reporters include a carboxy-terminal PEST signal that reduces the half-life of each reporter to less than 1 hour and dramatically improves the temporal resolution of these assays, allowing these reporters to serve as proxies for transcriptional activation. We are focusing on the characterization of the transcriptional expression patterns of representative genes that display distinctly pulsatile expression at each larval stage. These classes include genes whose transcripts that are dramatically upregulated immediately before *lin-42* expression.

STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

L. Joshua-Tor C. Ashar, D. Bahel, E. Elkayam, C. Faehnle, S. Goldsmith, E. Greene, C. Kuhn, C. Kuscu, T. Lavy, S.-J. Lee, D. Sau, T. Schalch, A. Tocilj, D. Wah, J. Walleshauser

We study the molecular basis of nucleic acid regulatory processes by using the tools of structural biology and biochemistry to examine proteins and protein complexes associated with these processes. X-ray crystallography enables us to obtain the three-dimensional structures of these molecular machines. Biochemistry and molecular biology allow us to study properties that can be correlated to protein structure and function.

Mechanisms of RNAi

T. Schalch, C. Faehnle, E. Elkayam, C. Kuhn, C. Kuscu, J. Ipsaro, C. Ashar, E. Greene [in collaboration with G.J. Hannon, R.A. Martienssen, Cold Spring Harbor Laboratory; 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 has become an extraordinarily 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 obtain a true mechanistic understanding of these pathways, we must understand how the components of the RNAi machinery worked at a molecular level. Therefore, we embarked on structural and biochemical studies of key proteins in the RNAi pathway.

During RNAi, long double-stranded RNA (dsRNA) is processed to yield short (~19–31 nucleotides) dsRNAs that trigger the RNAi response. These short RNAs become incorporated into effector complexes called RNA-induced silencing complexes (RISCs). In mature com- plexes of this type, a single-stranded RNA (ssRNA) that is the antisense strand of the original dsRNA is retained. This short RNA (a small interfering RNA [siRNA] or a microRNA [miRNA]) then acts to guide the RISC to its target through base complementarity. The best-characterized pathway, and the one that is predominantly used for gene knockdown technology, is a posttranscriptional genes silencing (PTGS) pathway called "slicing." Here, the RISC 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 RISCs. In all cases, these complexes contain a small ssRNA and an Argonaute protein, which serve to define the RISC. In the past few years, we have been studying Argonaute family proteins, their complexes, and their roles in various RNAi silencing pathways.

Proper segregation of chromosomes during cell division depends on kinetochore assembly, which is, in turn, dependent on heterochromatin assembly. In the fission yeast *Schizosaccharomyces pombe*, assembly of centrometic heterochromatin requires the RITS complex, a specialized RISC that physically anchors small noncoding RNAs to chromatin. It consists of the Argonaute protein Ago1, the chromodomain-containing protein Chp1, the novel protein Tas3, and siRNAs derived from centromeric repeats. Our crystal structure of Chp1's chromodomain in complex with a peptide corresponding to histone H3 with a trimethylated lysine 9, a histone mark characteristic of heterochromatin, revealed extensive sites of contact that contribute to Chp1's high-affinity binding. 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 (trimethylated lysine-9 H3 peptide) is greatly reduced. Apart from the chromodomain, Chp1 had no recognizable domains. Our structure of the carboxy-terminal half of Chp1 in complex with the Tas3 amino-terminal domain reveals Chp1's tight embrace of Tas3. It also reveals the presence of a PIN domain at the carboxyl terminus of Chp1, which contributes to posttranscriptional gene silencing of subtelomeric transcripts independently of RNAi. Our studies suggest that the Chp1-Tas3 complex provides a solid and versatile platform to recruit both RNAi-dependent and RNAi-independent gene-silencing pathways for locus-specific regulation of heterochromatin (Fig. 1).

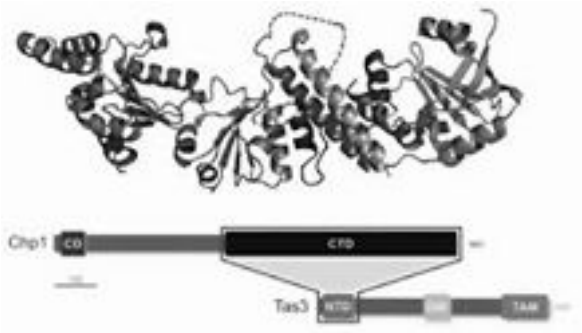


Figure 1. Structure of the Chp1-Tas3 complex. (*Top*) Structure of the Chp1-Tas3 core. (*Bottom*) Domain representation of Chp1 and Tas3 depicting the carboxy-terminal domain (CTD) of Chp1 defined by limited proteolysis and the amino-terminal domain (NTD) of Tas3. Also indicated are the chromodomain (CD) of Chp1, the GW domain, and the carboxy-terminal Tas3 α-helical motif (TAM).

The Different Faces of E1: A Replicative Hexameric Helicase

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. Our early studies focused on recognition of the DNA replication origin by the helicase. Our crystal structure of the replicative helicase E1 from papillomavirus bound to single-stranded DNA and nucleotide molecules at the ATP-binding sites provided a unique look into the mechanism of translocation of this molecular machine along the DNA.

We continue to study this helicase and the role other domains of the protein might have in helicase assembly and activity. We are also using biophysical studies in solution to understand the helicase in mechanistic detail and to correlate with the structural framework.

A Transcriptional Switch Revisited

T. Lavy, D. Wah [in collaboration with R. Sternglanz, Stony Brook University; S.A. Johnston, Arizona State University; J. Rabinowitz, Princeton University; A. Caudy, University of Toronto]

A wealth of genetic information and some biochemical analysis have revealed an elegant model for the *GAL* regulon of the yeast *Saccharomyces cerevisiae*, a beautiful model system to understand transcriptional activation in eukaryotes. While attempting to place these studies into a structural and biochemical framework, we discovered the involvement of a dinucleotide in the induction of this classical transcriptional switch. Specifically, nicotinomide adenine dinucleotide phosphate (NADP) appears to jump-start the switch, which is then sustained by the previously known transducer-repressor (Gal3p-Gal80p) interaction. This represents a real shift in how we think about the *GAL* transcriptional switch and our textbook view of how these switches work. In light of this discovery, we would like to find the link between galactose induction and the metabolic state of the cell that is sensed by the repressor Gal80p. Our goal is to further delineate the molecular interactions and possible enzymatic activities involved in induction, activation, and repression in order to provide a mechanistic view of this switch. We are using a combination of X-ray crystallography, biochemistry, genetics, and metabolomics to more fundamentally understand this biological paradigm.

The most recent addition is our structure of the complex between the Gal80p repressor, the Gal3p transducer, and the two small-molecule ligands galactose and ATP (Fig. 2). We have found that the interaction between the

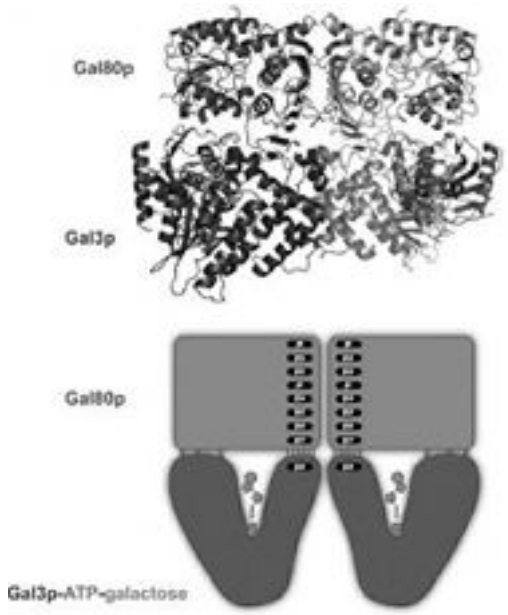


Figure 2. The structure of the Gal80p-Gal3p-ATP-galactose heterotetramer complex. (*Top*) Ribbon diagram of the complex of a dimer of Gal80p and a dimer of Gal3p is shown with the ATP and galactose ligands. ATP and galactose are shown in stick representations. (*Bottom*) A schematic representation of the Gal80p-Gal3p-ATP-galactose heterotetramer complex illustrating the mode of binding. Gal3p binds Gal80p in a closed conformation with a β-strand from Gal3p extending the large β-sheet that forms the Gal80p dimerization interface.

proteins occurs only when Gal3p is in a "closed state" induced by ligand binding. Comparison to the free Gal3p structure that we have also determined, and a previously determined structure of a galactose and ATP-bound form of the very similar active galactokinase, Gal1p, clearly shows that ligand binding induces the closed state of Gal3p. Furthermore, examination of the sites of Gal3p constitutive mutations shows that these would favor a closed state for Gal3p without necessitating ligand binding. On the other hand, Gal80p superrepressor mutants appear to result from their inability to form a stable complex with Gal3p, rather than tighter association with the activator Gal4p.

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

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In Press

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Tali Lavy

RNA SPLICING

A.R. Krainer M. Akerman, O. Anczuków, I. Aznárez, F. Bezirci, L. Chartarifsky, D. Chatterjee, S. Das, O. Fregoso, Y. Hua, M. Jensen, H.-Y. Jeon, K.-T. Lin, Y. Liu, Y.-H. Liu, C. Long, L. Manche, J. Novatt, X. Roca, K. Sahashi, R. Sinha, R.-Y. Tzeng, Z. Wang, J. Wu, A. Xu, L. Zepeda

Mechanisms of Constitutive and Alternative Pre-mRNA Splicing

RNA splicing is required for 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, >90% 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 five noncoding 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 various roles 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.

Position-Dependent Splicing Activation and Repression by RBFOX1 and RBFOX2

RBFOX1 and RBFOX2 are alternative splicing factors that are predominantly expressed in brain and skeletal muscle. They specifically bind the RNA element UGCAUG and regulate alternative splicing positively or negatively in a position-dependent manner. The molecular basis for the position dependence of these and other splicing factors on alternative splicing of their targets is not known. We explored the mechanisms of RBFOX splicing activation and repression using a tethering assay via bacteriophage MS2 coat protein. We found that the Ala/Tyr/Gly-rich carboxy-terminal domain of RBFOX1/2 is sufficient for exon activation when tethered to the downstream intron, whereas both the carboxy-terminal domain and the central RRM (RNA-recognition motif) are required for exon repression when tethered to the upstream intron, thus recapitulating the position-dependent properties of the intact protein. Using immunoprecipitation and mass spectrometry, we identified hnRNP H1, Raly, and TFG as proteins that specifically interact with the carboxy-terminal domain of RBFOX1 and RBFOX2. RNA interference (RNAi) experiments showed that hnRNP H1 and TFG modulate the splicing activity of RBFOX1/2, whereas Raly had no effect. However, TFG is localized in the cytoplasm and likely modulates alternative splicing indirectly.

Alternative Splicing and Cancer

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 charac-

teristic of all cancer cells, known as the Warburg Effect. Cancer cells preferentially metabolize glucose by aerobic glycolysis, characterized by increased lactate production. This distinctive metabolism involves reexpression 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 isozymes are expressed from a single *PK-M* gene through alternative splicing of a pair of mutually exclusive exons (exons 9 and 10, respectively). In collaboration with Lewis Cantley (Harvard Medical School) and Matthew Vander Heiden (Massachusetts Institute of Technology), we demonstrated that *PK-M* is regulated by reciprocal effects on alternative exon use, such that exon 9 is repressed and exon 10 is activated in cancer cells. We further showed that exonic, rather than intronic, *cis* elements are the key determinants of *PK-M* splicing isoform ratios. Using a systematic subexonic duplication approach, we identified a potent exonic splicing enhancer in exon 10, which differs from its homologous counterpart in exon 9 by only two nucleotides. We identified SRSF3 as one of the cognate factors and showed that this SR protein activates exon 10 and mediates changes in glucose metabolism. These findings provided mechanistic insights into the complex regulation of alternative splicing of a key regulator of the Warburg Effect, and they also have implications for other genes with a similar pattern of alternative splicing.

SRSF1 (formerly SF2/ASF) is a prototypical serine- and arginine-rich (SR) protein with important roles in splicing and other aspects of mRNA metabolism. We previously determined that SRSF1 is a potent oncoprotein with increased expression in many tumors, including breast cancer. In collaboration with Senthil Muthuswamy (Ontario Cancer Institute) and Rotem Karni (Hebrew University, Jerusalem), we have investigated SRSF1's ability to transform human and mouse mammary epithelial cells in vivo and in vitro. We showed that SRSF1-overexpressing mouse COMMA-1D cells form tumors after orthotopic transplantation to reconstitute the mammary gland. In organotypic three-dimensional (3D) culture, SRSF1-overexpressing human MCF-10A cells form larger acini than control cells, reflecting increased proliferation and delayed apoptosis during acinar morphogenesis. We found that these effects require the first RNA-recognition motif and nuclear functions of SRSF1. SRSF1 overexpression promotes alternative splicing of *BIM* and *BIN1* isoforms that lack proapoptotic functions and contribute to the acinar phenotype. We also demonstrated that *SRSF1* cooperates specifically with *MYC* to transform mammary epithelial cells, in part by potentiating activation of the translation initiation factor oncoprotein eIF4E. Furthermore, *MYC* and *SRSF1* show correlated expression in human breast tumors.

SRSF1 is also up-regulated in lung carcinomas, where it also shows significant correlation with MYC expression. We have now demonstrated that *SRSF1* is a direct transcriptional target of MYC, providing a mechanistic basis for their correlated expression in cancer. MYC knockdown down-regulates *SRSF1* expression in multiple lung cancer cell lines. MYC directly activates transcription of *SRSF1* through two noncanonical E-boxes in its promoter. The resulting increase in SRSF1 protein is sufficient to modulate alternative splicing of a subset of its target transcripts. In particular, MYC induction leads to SRSF1-mediated alternative splicing of the signaling kinase *MKNK2*. We further showed that SRSF1 knockdown reduces MYC's oncogenic activity, decreasing proliferation and anchorage-independent growth. These results suggest a mechanism for SRSF1 up-regulation in a subset of tumors with elevated MYC levels and identify *SRSF1* as a critical MYC target that contributes to its oncogenic potential by enabling MYC to regulate the expression of specific protein isoforms through alternative splicing.

Targeted Antisense Modulation of Alternative Splicing for Therapeutic Purposes

Spinal muscular atrophy (SMA) is a common, autosomal-recessive motor neuron degeneration 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. We have developed an antisense method to increase the extent of exon 7 inclusion during splicing of *SMN2* transcripts, for eventual therapeutic use in SMA. This translational research is being done in collaboration with Frank Bennett and colleagues (Isis Pharmaceuticals, California).

There is no effective treatment for SMA, but restoring correct SMN expression in spinal cord motor neurons was expected to be necessary and perhaps sufficient.

However, pathologies outside of the nervous system (CNS), including cardiovascular defects, were recently reported in severe SMA mouse models and patients, reflecting autonomic dysfunction or direct effects in cardiac tissues. We compared systemic versus CNS restoration of SMN in a severe mouse model. We used an antisense oligonucleotide (ASO), ASO-10-27, that effectively corrects *SMN2* splicing and restores SMN expression in motor neurons after intracerebroventricular injection. Systemic administration of ASO-10-27 to neonates robustly rescued severe SMA mice, much more effectively than intracerebroventricular administration; subcutaneous injections extended the median life span by 25-fold. Furthermore, neonatal SMA mice had decreased hepatic *Igfals* expression, leading to a pronounced reduction in circulating insulin-like growth factor 1 (IGF-1), and ASO-10-27 treatment restored *Igfals* mRNA and circulating IGF-1 protein to normal levels. These results suggest that the liver is important in SMA pathogenesis, underscoring the importance of SMN in peripheral tissues, and demonstrate the remarkable efficacy of a promising drug candidate. This antisense compound already entered Phase I clinical trials in December.

Splice Site Recognition and Human Genetics

We continued to analyze the relationship between splice site mutations and genetic diseases. In 1997, we discovered a very rare class of introns with noncanonical splice sites that are nevertheless recognized by the major spliceosome, and we characterized one such intron in a voltage-gated sodium channel gene, *SCN4A*. Masanori Takahashi (Osaka University) recently identified an insertion/deletion mutation in this intron in a myotonia patient, and we collaborated with his group to characterize the nature of the splicing defect responsible for the phenotype. The mutation prevents correct recognition of the 5′ splice site, resulting in activation of cryptic splice sites. This gives rise to a 35-amino-acid insertion in the sodium channel, which functions abnormally and causes muscle disease.

Quantifying Alternative Splicing by Next-Generation Sequencing

Alternative splicing is a pre-mRNA maturation process leading to the expression of multiple mRNA variants from the same primary transcript. More than 90% of human genes are expressed via alternative splicing. Therefore, quantifying the inclusion level of every exon is crucial for generating accurate transcriptomic maps and studying the regulation of alternative splicing. In collaboration with Michael Zhang (University of Texas, Dallas), we developed SpliceTrap, a method to quantify exon inclusion levels using paired-end RNA-Seq data. Unlike other tools, which focus on full-length transcript isoforms, SpliceTrap approaches the expression-level estimation of each exon as an independent Bayesian inference problem. In addition, SpliceTrap can identify major classes of alternative splicing events under a single cellular condition, without requiring a background set of reads to estimate relative splicing changes. We tested SpliceTrap both by simulation and real data analysis and compared it to state-of-the-art tools for transcript quantification. SpliceTrap demonstrated improved accuracy, robustness, and reliability in quantifying exon-inclusion ratios.

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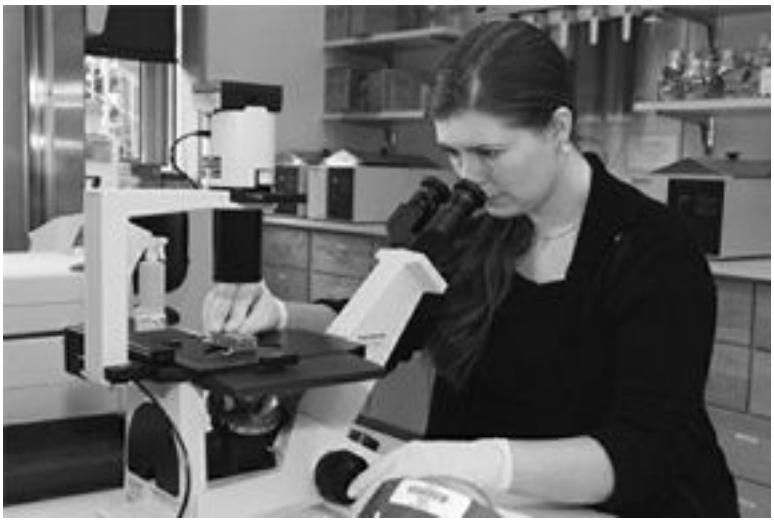

Olga Anczuków-Camarda

CELL BIOLOGY OF THE NUCLEUS

D.L. Spector G. Arun, J. Bergmann, M.S. Bodnar, M. Eckersley-Maslin, S. Hearn, M. Hübner, R.I. Kumaran, Z. Lazar, J. Li, Y. Mao, C. Zepeda-Mendoza, 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, some of which encode for proteins, whereas others are functional in the form of RNA. Although much biochemical information is available regarding many of the factors involved in gene expression, the spatial and temporal parameters that influence gene expression and the role of noncoding RNAs in regulating gene expression are just beginning to be elucidated. During the past year, our research has focused on two main areas: (1) examining the nuclear organization of mouse embryonic stem cells and transitions to neural progenitor cells and (2) developing and characterizing a loss-of-function mouse model of a long nuclear-retained noncoding RNA.

Organization and Expression of Genes in Embryonic Stem Cells and Neural Progenitor Cells

M. Eckersley-Maslin, M. Bodnar, J. Bergmann, Z. Lazar

We are interested in developing a complete understanding of the spatial and temporal events involved in the regulation of gene expression in embryonic stem cells (ESCs) as they transit from the pluripotent state to the differentiated state. Such an understanding will provide important insight into the underlying principles of nuclear organization and gene expression that will be critical to fully reveal the signals that regulate the transition from the pluripotent state to the differentiated state. Pluripotent ESCs are characterized by an extremely dynamic chromatin organization and nuclear morphology. The irregular nuclear shape of the ESC contains many invaginations and protrusions, which are highly dynamic. Upon differentiation, this nuclear architecture transitions to a smooth and rigid form, coupled with a decrease in the open chromatin configuration, suggesting a tight connection between nuclear structure and the differentiation program. The major structural component of the nuclear periphery is the nuclear lamina that is composed of lamin proteins, which also have important functions in gene regulation and chromatin organization. Loss of lamin proteins results in severe developmental defects, and mutation of *Lamin A/C* is associated with premature aging in individuals with progeria (for review, see Dechat et al., *Cold Spring Harb Perspect Biol 2:* a000547 [2010]). Although ESCs express lamin B1 and B2, lamin A/C has been reported to be completely absent in ESCs, with levels increasing during embryogenesis (Constantinescu et al., *Stem Cells 24:* 177 [2006]). This purported lack of lamin A/C in ESCs has served as a landmark for models and hypotheses explaining pluripotency, chromatin dynamics, and ESC nuclear plasticity (for review, see Mattout and Meshorer, *Curr Opin Cell Biol 22:* 1 [2010]).

We sought to systematically reinvestigate whether ESCs express lamin A/C. Unexpectedly, we found that both *lamin A* and *lamin C* transcripts in ESCs were readily detected by quantitative reverse transcriptase–polymerase chain reaction (q-RT-PCR) at a level similar to that of the neural progenitor cells (NPCs). Examination of whole-genome poly(A)$^+$ RNA-sequencing data, as well as published data sets from mouse and human ESCs, confirmed that full-length *lamin A/C* mRNA was expressed. Immunoblotting using three independent antibodies demonstrated that *lamin A/C* mRNA transcripts were efficiently translated into both protein isoforms. As a control, no signal was seen in a *lamin A* knockout (lmna$^{-/-}$) ESC line, confirming antibody specificity and purity of the ESCs from mouse embryonic fibroblast (MEF) feeder cells. Both AB2.2 and lmna$^{-/-}$ ESCs expressed the pluripotency marker Oct4. Lamin A/C was also detected in three early-passage ESC lines, four additional well-established ESC lines, and an iPS cell line. Finally, expression was validated in individual cells by immunofluorescence. Lamin A/C was detected at the nuclear periphery of all Oct4-positive ESCs but not in lmna$^{-/-}$ ESCs. Correct

localization was further confirmed in additional ESC lines and also with an independent antibody. Notably, ESCs have significantly lower levels of Lamin A/C when compared to MEFs, which may explain why previous reports have failed to detect Lamin A/C in ESCs.

In addition to examining the organization of the nuclear periphery in ESCs, we have also initiated an analysis of the dynamics of specific genes that change their expression profile as cells transit from the pluripotent state to the differentiated state. We have performed RNA sequencing screens to identify candidate protein-coding genes and genes encoding noncoding RNAs (ncRNAs) that change their expression levels upon differentiation. We are currently in the process of characterizing and prioritizing these candidates, and during the next year, we will perform a series of molecular and cell biological studies to identify interesting mechanisms of gene regulation.

Generation and Characterization of a Knockout Mouse Model Lacking Malat1, an Abundant Long Noncoding RNA

B. Zhang, G. Arun, Y. Mao, Z. Lazar [in collaboration with C. Zhang, The Rockefeller University]

Genome-wide studies during the last 5 years have indicated that the majority of the human and mouse genomes are transcribed, yielding a complex network of transcripts that include tens of thousands of ncRNAs with no protein-coding capacity (for review, see Orom and Shiekhattar, *Curr Opin Genet Dev 21:* 194 [2011]). Long noncoding RNAs (lncRNAs), the largest and most complex class of ncRNAs, are mRNA-like RNA polymerase II transcripts ranging in size from 200 nucleotides to >100 kb in various cell types (for review, see Wilusz et al., *Genes Dev 23:* 1494 [2009]). The majority of lncRNAs are expressed at very low levels, some as low as one copy per cell, and these RNAs generally exhibit poor primary sequence conservation over evolution. lncRNAs have been implicated in numerous molecular functions, including modulating transcriptional patterns, regulating protein activities, serving structural or organizational roles, altering RNA processing events, and serving as precursors to small RNAs (for review, see Wilusz et al., *Genes Dev 23:* 1494 [2009]).

Malat1 (metastasis-associated lung adenocarcinoma transcript 1), also known as *Neat2* (noncoding nuclear-enriched abundant transcript 2), is located on mouse chromosome 19qA (human chromosome 11q13.1), is evolutionarily conserved among mammals, and is highly expressed in many tissues. *MALAT1* is also up-regulated in several human cancers, suggesting that it may have an important function during development and cancer progression. Previous studies in cell lines have indicated proliferative (Yang et al., *Cell 147:* 773 [2011]) as well as antiproliferative (Tripathi et al., *Mol Cell 39:* 925[2010]) functions of MALAT1. However, the physiological function of *Malat1* lncRNA at the tissue and organismal levels has not been investigated.

To assess the in vivo function of mouse *Malat1*, we established a loss-of-function mouse genetic model using homologous recombination in ES cells. We found the recombination rate at the *Malat1* locus to be 43%, much higher than other loci in the genome. The mutant mice, lacking a 3-kb 5′ region of the *Malat1* gene including its promoter region, were genotyped by Southern blotting and tail DNA PCR analyses. Northern blotting results showed that the *Malat1* transcript is completely depleted in $Malat1^{-/-}$ tissues, indicating that $Malat1^{-}$ is a null allele. $Malat1^{-/-}$ mice are grossly normal and fertile. Offspring of heterozygous breedings follow Mendelian segregation, suggesting that deletion of *Malat1* does not affect mouse prenatal and postnatal viability. Breedings between *Malat1* homozygotes and wild type produce normal-sized litters, indicating that $Malat1^{-/-}$ mice are fertile. Further cell biological and biochemical analyses indicated that mouse *Malat1* is not essential for nuclear speckle assembly, regulating the level and phosphorylation of SR splicing factors, or cell proliferation.

To assess the level of *Malat1* lncRNA in mouse liver and brain cortex, we performed RNA-Seq profiling and obtained ~300 million paired-end reads for mouse liver and brain cortex. Reads from protein-coding genes, known housekeeping regulatory RNAs, small RNA precursors, and repeats were filtered out to produce lncRNA profiles. *Malat1* comprised ~15% of the lncRNA sequence reads in liver. The reads per kilobase million (RPKM) for *Malat1* in liver are extremely high as compared to several housekeeping protein-coding genes (*Malat1*, 109 RPKM; β-*Actin*; 177 RPKM; *Gapdh*, 21 RPKM). Genome-wide expression and splicing profiling demonstrated that *Malat1* loss results in minimal alterations in global gene expression and pre-mRNA splicing. However, deletion of *Malat1* resulted in a nearly twofold up-regulation of a small group of genes in the mouse brain cortex. Many of these genes reside within a 230-kb region of the *Malat1* gene, indicating a potential *cis* regulatory role of *Malat1*

gene transcription. Ongoing studies will assess the potential role of the *Malat1* gene locus in regulating the expression of these genes.

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David Spector, R. Ileng Kumaran

MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

A. Stenlund S. Schuck

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

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

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

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

Phosphorylation by the Protein Kinase CK2 Regulates the DNA-Binding Activities of the Papillomavirus E1 and E2 Proteins

The viral E1 and E2 proteins are site-specific DNA-binding proteins that recognize specific binding sites in the viral genome and together control viral gene expression and viral DNA replication. E2 binds to multiple binding sites in the E2-dependent enhancer and activates or represses viral gene expression, whereas E1 binds to a small cluster of sites in the origin of DNA replication (ori) and prepares the ori for initiation of DNA replication. E1 also functions as the replicative DNA helicase. The viral life cycle consists of at least two distinct stages. In the latent stage, the early viral genes are expressed and the viral DNA is replicated at a low level. In the vegetative state, the late viral genes (such as the capsid proteins) are expressed, the viral DNA is replicated at a very high level, and new virus particles are assembled. It is believed that the transition

from the latent stage to the vegetative stage is controlled by the differentiation of the infected cells and likely involves changes in cellular gene expression. However, the precise signals that trigger the switch to vegetative replication are unknown.

We wanted to determine whether phosphorylation of the viral E1 protein has a role in the viral life cycle. It is well established that E1 can be phosphorylated by the protein kinase CK2 in vitro. To determine what effects CK2 phosphorylation has on E1, we subjected recombinant E1, purified from *Escherichia coli*, to CK2 phosphorylation and tested the protein for various in vitro assays related to initiation of DNA replication. Interestingly, CK2 phosphorylation of E1 resulted in the loss of site-specific DNA-binding activity. To map the inactivating phosphorylation sites, we tested the amino-terminal half of E1 ($E1_{1–308}$), which contains the E1 DNA-binding domain (E1 DBD), for binding with or without phosphorylation. This fragment was also inactivated for DNA binding, demonstrating that the inactivating phosphorylation sites were present in the amino-terminal half of E1. In collaboration with C. Ruse (CSHL Proteomics Facility), we performed phosphorylation analysis of CK2-phosphorylated E1 by mass spectrometric analysis and identified multiple phosphorylation sites in the amino-terminal domain, each of which was individually capable of inactivating E1 DNA binding. Interestingly, none of these phosphorylation sites were present within the E1 DBD, indicating that phosphorylation events outside of the E1 DBD inactivate DNA binding. Consistent with this result, the isolated E1 DBD ($E1_{142–308}$) could not be inactivated by CK2 phosphorylation. The inactivation of DNA binding by CK2 phosphorylation is conserved also in the E1 proteins from HPV-11 and HPV-31, indicating that the mechanism is conserved for other papillomavirus E1 proteins.

Inspired by this result, we next examined the effect of CK2 on the viral E2 protein. Previous studies have indicated that CK2 phosphorylation affects the half-life of E2. Instead, we found that similar to the effect on E1, CK2 phosphorylation resulted in complete loss of E2 DNA-binding activity. Also as in E1, the two phosphorylation sites responsible for the inactivation of DNA binding are located outside the E1 DBD, demonstrating that the inactivation of the DBD likely involves structural rearrangements.

To determine precisely what role CK2 phosphorylation of E1 and E2 has in the viral life cycle, we mutated all of the CK2 sites in the amino terminus of E1 and the two sites in E2 in the context of the papillomavirus genome. We then tested the mutant genomes for viral DNA replication and transformation in a cell culture system that represents the latent stage of the viral life cycle. The phosphorylation site mutations in E2 showed greatly increased levels of viral DNA replication and also increased the morphological transformation as measured by focus formation, indicating that phosphorylation at these sites has an important role in the viral life cycle. Surprisingly, however, the phosphorylation mutations in the amino terminus of E1 had no obvious phenotype. Because the DNA-binding inactivation is conserved, the lack of a clear phenotype of these E1 mutations likely is due to the fact that only a part of the viral life cycle (the latent stage) is reproduced in these assays.

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DNA REPLICATION AND CHROMATIN INHERITANCE

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

Our genome contains the genetic information that is transmitted from one cell to its two daughter cells during cell proliferation and development of tissues and organs. The genetic information in DNA is also copied, and half of the duplicated information is passed on from a parent to a child. The process of duplicating the DNA double helix—the process central to genome inheritance—is therefore of fundamental importance and has been the focus of research in this laboratory for 31 years at Cold Spring Harbor. DNA is wrapped up with proteins that form chromatin, and chromatin structures, which happen to be different in the various cell types in our body, are also inherited by daughter cells as we grow from a fertilized egg to produce the ~100 trillion cells in our body. Thus, the information transfer must be accurate and efficient. This year, progress has been made on understanding how inheritance of genetic information influences the fate of cells during development, how the origin recognition complex (ORC) cooperates with Cdc6 to recognize DNA, and how essential DNA replication proteins can be studied in the mouse.

Inheritance of Chromatin and Gene Silencing

Previous research from this laboratory had uncovered how the histone proteins are assembled during the process of DNA replication. Each sister chromatid, the products of replicating a DNA molecule, must have its complement of histone proteins copied along with the DNA. The multisubunit chromatin assembly factor-1 (CAF-1) was shown to bind histones and load a dimer pair of newly synthesized histones H3/H4 onto DNA to form a tetramer that then recruits two dimers of the H2A/H2B histones to form a nucleosome containing eight histones $(H2A, H2B, H3, H4)_2$ and DNA. This process occurs immediately after the DNA replication fork copies the double helix of DNA and de novo nucleosomes can be assembled on both sister chromatids, although in cells, the parental H3/H4 tetramers are inherited. Thus, the two sisters end up with half the H3/H4 tetramers in new nucleosomes coming from the parental chromatin and half synthesized de novo. Because histone modifications can influence gene expression and chromosome structure, this inheritance may be important for developmental patterning.

This year, we hosted a graduate student from Robert Horvitz's laboratory at the Massachusetts Institute of Technology to perform some biochemical studies related to CAF-1-mediated chromatin assembly. Shunji Nakano had isolated mutants of the worm *Caenorhabditis elegans* that failed to establish an asymmetric neuronal cell fate during development. Normally during development, a cell called ABaraap divides four times to produce 16 cells, two of which die via programmed cell death and two become bilaterally asymmetric, with one cell on the left side of the body becoming an epithelial cell and its symmetrical distant cousin on the right side of the body becoming an M1 motor neuron. The mutants that failed to establish the motor neuron, and instead produced an additional epithelial cell in its place, mapped to a defective histone H3 gene, one of 24 genes encoding histone H3 in the worm genome. Biochemical characterization of the histone H3 mutant showed that it was a dominant inhibitor of the CAF-1-mediated DNA replication-dependent chromatin assembly. Furthermore, Nakano and Horvitz showed that depletion of CAF-1 subunits during worm development yielded the same defect in bilateral asymmetry of the M1 motor neuron. These results have significant implications for the mechanism of cell fate determination, particularly of bilateral asymmetry. Because the known transcription factors that determine the M1 motor neuron cell fate were not altered by the histone H3 or CAF-1 mutations, it is likely that a chromatin structure is established during DNA replication in S phase of the M1 mother cell so that one sister chromatid is marked and transmitted to the M1 postmitotic cell, thereby allowing a neuronal fate to be established.

In previous years, we had shown that CAF-1 mediated nucleosome assembly during DNA replication required an interaction between CAF-1 and the prolifer-

ating cell nuclear antigen (PCNA), a DNA polymerase clamp. PCNA is loaded and unloaded onto the DNA every time a DNA polymerase starts synthesis, and thus, because of the discontinuous synthesis of Okazaki fragments on the lagging strand versus continuous DNA synthesis on the leading strand of a DNA replication fork, the two sister chromatids will have different amounts of PCNA topologically linked to them before it is unloaded. The strand bias of PCNA therefore may translate into a strand bias in the nature of nucleosomes assembled on the leading- and lagging-strand products. Such a strand bias could provide asymmetry in the two sister chromatids that are inherited by the daughter cells, leading to bilateral asymmetry of cell fate. It is interesting to compare the phenotype in the specific lineage in *C. elegans* and the phenotype of CAF-1 mutants in the plant *Arabidopsis* that produce plants with highly variable, epigenetically determined cell fates.

Conditional Depletion of Essential Genes in Mice

During a screen to identify new DNA replication proteins in human cells using Greg Hannon's (CSHL) short hairpin RNA (shRNA) library, we identified genes that when inhibited could completely block the proliferation of all human cells. Surprisingly, depletion of substantial amounts of known DNA replication proteins, even in some cases up to 80%–90%, allowed some cell proliferation of cells in culture. In the unbiased screen using Hannon's version-3 shRNA library and some custom-made shRNAs, we identified multiple shRNAs that depleted either subunits of replication protein A (RPA) or ribonucleotide reductase as being the most potent in inhibiting cell proliferation. RPA is a protein that we and other investigators identified in the late 1980s as an essential DNA replication, repair, and recombination protein that is also the primary sensor of single-stranded DNA for checkpoint signaling Thus, depleting this protein compromises multiple processes in the cell and prevents checkpoint signaling, blocking progression through mitosis and thereby enhancing cell death.

In collaboration with Scott Lowe's laboratory at CSHL, we showed that shRNAs directed to the smallest subunit of RPA, the RPA3 protein, blocked tumor cell proliferation in vivo. We also made several versions of mice in which the RPA3 shRNAs were conditionally expressed in all tissues from doxycycline-inducible promoters by feeding the mouse doxycycline in its drinking water. Thus, mice could be bred and grown to adulthood before adding doxycycline to allow for normal development and cell proliferation, but when the shRNAs were induced in adult mice, they lost weight rapidly due to loss of cell proliferation in gut epithelial cells. Removal of the doxycycline turned off the transgene and the mice could recover the weight loss, with concomitant proliferation of the gut epithelial cells. Thus, this inducible genetic system allows for the conditional expression of any gene and thereby provides the ability to examine the phenotype of depleting essential proteins in vivo. It also offers the opportunity to assess the side effects of drug targets in adult mice if shRNAs directed to the drug target are conditionally expressed in all tissues.

Origin Recognition Complex

ORC is a key protein that cooperates with Cdc6 and Cdt1 to load a double hexamer of the MCM2-7 complex onto origin DNA to form a pre-replicative complex (pre-RC). Pre-RC assembly happens during mitotic exit or during G_1 phase and is a precursor to the initiation of DNA replication in S phase. In collaboration with Huilin Liu at Brookhaven National Laboratory (BNL) and former postdoctoral fellow Christian Speck at the MRC Laboratory at Hammersmith Hospital in London, we have determined the cryo-electron microscopy (cyro-EM) structure of ORC bound to an origin DNA and of the ORC-Cdc6 complex bound to origin DNA (see Fig. 1). The ORC-DNA structure shows that ORC is a flat, crescent-shaped structure that has the Orc1 amino terminus containing the BAH (bromo adjacent homology) domain located in the center of the crescent. In contrast, the ORC-Cdc6-DNA structure, obtained at higher resolution than the ORC-DNA structure, shows that Cdc6 occupies the position previously occupied by the BAH domain, pushing it to the back of the structure. The ORC-Cdc6 on DNA forms a flat ring shape with many of the subunits visible as taking on the structure of typical AAA^+ proteins. New density, possibly Orc6 that has moved from the middle of the ORC crescent, is protruding from the front of the ring. Interestingly, Steve Bell's laboratory at MIT has shown that Orc6 binds two molecules of Cdt1, possibly explaining how the protruded Orc6 might recruit two MCM2-7 hexamers. The ORC-Cdc6 structure of DNA was of sufficient clarity that we could model into the cryo-EM structure the X-ray crystal structure of the AAA^+ Orc1/Cdc6 protein from an archaeal species. The Orc1/Cdc6 protein from

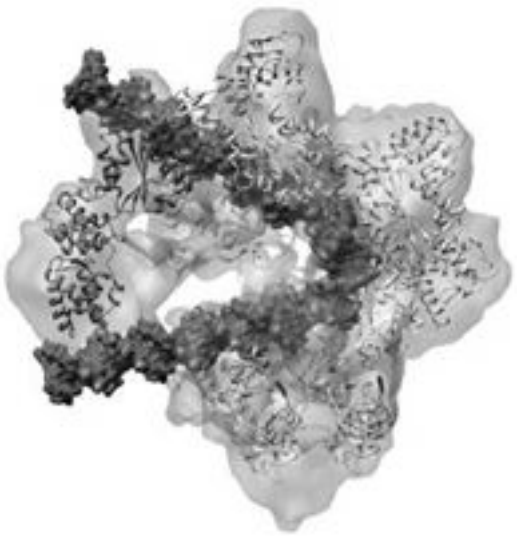

Figure 1. Model of the yeast origin recognition complex complexed with double-stranded DNA incorporating a sequence that encodes the replication origin. The 3D structure of the complex with bound DNA was determined by single-particle cryo-electron microscopy in collaboration with Dr. Huilin Li at Stonybrook University and Brookhaven National Laboratory. The surface envelope is shown. The locations of the ORC subunits are shown, and superimposed molecular structures are models of the ORC AAA^+ subunits (determined by X-ray crystallography for a related archaeal protein). The ORC is a six-protein machine with a slightly twisted half-ring conformation that is proposed to wrap around and bend ~70 bp of double-stranded DNA. When a replication initiator protein Cdc6 (left-hand side) binds ORC, the ring is now complete and ready to load the MCM2-7 hexamer protein onto the DNA. The DNA is modeled into the determined ORC-Cdc6 protein structure.

archaea is the primary origin recognition protein and itself forms a C-shaped molecule when bound to DNA. We proposed that five of the six ORC subunits (all but Orc6) form a C-shaped structure similar to the archaeal proteins when bound to DNA. Although we could not see the DNA density in the cryo-EM structure of ORC-Cdc6, we have a good working model of how ORC and Cdc6 bind to DNA.

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CANCER: GENETICS

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

Gregory Hannon is a pioneer in the study of RNA interference (RNAi), a process by which double-stranded RNA molecules induce gene silencing. Hannon and colleagues have elucidated key elements of RNAi machinery and have led the way in using RNAi to study cancer biology and genetics, generating libraries of short-hairpin RNAs (shRNAs) that have been widely applied in gene silencing studies. During the past several years, the Hannon lab has focused on roles of small RNAs in germ cells, which tend to have the most elaborate set of small RNA pathways of any cell type. They have discovered an essential role for pseudogenes in producing small RNAs that are critical for proper oocyte development and an elegant small-RNA-based immune system that guards the genome against transposable elements. The Hannon lab also strives to understand the biology of cancer cells, with a focus on breast and pancreatic cancer. Hannon lab scientists are exploring the roles of small RNAs as oncogenes and tumor suppressors and are exploiting the RNAi libraries that they have developed to identify new therapeutic targets for specific disease subtypes. Additionally, they are taking genetic approaches to understand the biology of resistance to currently used targeted therapies. Another research thrust of the Hannon team is to exploit the power of next-generation sequencing to understand the biology of the mammalian genome. Their efforts range from the identification of new classes of small RNAs to understanding human evolution and diversity. In the last year, they have placed a major emphasis on the evolution of the epigenome and its role in driving cell-fate specification.

Scott Lowe's lab studies cancer gene networks and determines how genetic lesions affecting these networks contribute to tumor development and resistance to therapy. Lowe's team has adapted RNAi technology to produce animal models in which genes can be switched on and off in a spatial, temporal, and reversible manner, and they have used this to study the impact of tumor suppressor genes on tumor development and maintenance. The team has also spearheaded a collaborative effort involving other CSHL labs to identify novel tumor suppressor genes. They combined their earlier integrated oncogenomics approach to human liver cancers with RNAi-based screening in a mouse model. Lowe's lab also studies cellular senescence, a potentially powerful mechanism for suppressing tumor formation. Lowe lab investigators have discovered that the gene-suppressing activity of the *retinoblastoma* (RB) gene can be traced to its ability to trigger senescence. The team has also demonstrated that senescence helps to limit wound-healing responses in liver disease, a finding that suggests that growth arrest has functional relevance, in addition to its role in 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, which have proven to be 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 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, aging, and autism, identifying the genetic players and determining how aberrations in their functions culminate in human disease. Through innovative use of a technique called "chromosome engineering," this year the Mills group has demonstrated that one of the most common genetic alterations in autism—deletion of a 27-gene cluster on chromosome 16—causes autism-like features in mice. The mouse models also revealed a

potential link between the 16p11.2 deletion and survival because about half of the mice died following birth. Previously, Mills used chromosome engineering to identify 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. Mills lab members succeeded in halting the growth of malignant tumors by turning on production of one of the proteins, called TAp63, that is encoded by the *p63* gene. During the last year, they discovered that a different version of p63, called ΔNp63, reprograms stem cells of the skin to cause carcinoma development—the most prevalent form of human cancer. Modulation of these proteins may offer new methods of treatment for human malignancies.

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 to liver, colon, and lung cancer. This past year, Powers and colleagues identified a strategy for targeted molecular therapy in liver cancer based on tumor dependency after amplification of the *FGF19* oncogene. Powers has also had an important role in the development of a distinctive CSHL approach to the functional study of cancer genes. Called integrative oncogenomics, it is a rapid large-scale screen for genes that are deleted or amplified in human cancers and suspected of being tumor suppressors, in the case of deletions, or oncogenes, in the case of amplifications.

Michael Wigler, with James Hicks and others, studies human cancer and genetic disorders from a population genomics perspective. Their cancer effort focuses on breast and prostate cancer (the latter jointly with Lloyd Trotman) and involves collaborative clinical studies (with local, national, and international collaborators) to discover mutational patterns predicting treatment response and outcome. The lab also develops methodology for single-cell genomic and RNA analysis aimed at detecting cancer cells in bodily fluids such as blood and urine. This has led to major potential applications for the early detection of cancer and to monitoring its recurrence and response to therapy during and after treatment. Single-cell analysis has also led to insights about the clonal evolution and heterogeneity of cancers and may lead to a better understanding of initiation, progression, and metastasis, as well as shed light on the stem cell hypothesis of cancer and host responses. The Wigler lab's genetic efforts are largely focused on determining the role of de novo mutation in pediatric disorders with a strong genetic contribution. They study autism as part of a large study of simplex families organized by the Simons Foundation, and they also study congenital heart disease and pediatric cancers with collaborators at, respectively, Columbia University and Memorial Sloan-Kettering Cancer Center. In collaboration with Alea Mills, they helped to create a mouse model for one of the most common genetic abnormalities contributing to autism. Recent work has confirmed and extended the team's previous observations on the role of de novo copy-number variation in autism, with similar results in the other disorders. In a large-scale exome sequencing project with W. Richard McCombie here at CSHL and with the Genome Sequencing Center at Washington University in St. Louis, the team has proven the role of small-scale de novo mutations that disrupt genes in autism. Results overall confirm their previous genetic models for autism incidence and identify strong candidate mutational targets from which biological models of autism can be made and tested.

RNA INTERFERENCE MECHANISMS AND APPLICATIONS

G. Hannon

M. Baer, S. Boettcher, D. Bressan, R. Burgess, A. Canela, K. Chang, S. Cheloufi, S. Chen, S.W. Chi, B. Czech, C. dos Santos, D. Fagegaltier, I. Falciatori, S. Fenoglio, W.S. Goh, P. Guzzardo, A. Haase, J. Hahn, E. Harrison, E. Hodges, I. Ibarra, F. Karginov, S. Knott, M. Kudla, E. Lee, Y. Luo, M. Malave, K. Marran, J. McGinn, A. Molaro, M. Mosquera, F. Muerdter, F. Pelon, C. Peterson, E. Piccinin, J. Preall, C. Rebbeck, F. Rollins, A. Rosebrock, E. Rozhkova, N. Rozhkov, L. Sabin, S. Shaw, E. Tonin

My lab continues to focus on three distinct areas. First, we study RNA biology, with a focus on noncoding RNAs. Second, we study the roles of small RNAs in cancer, mainly breast cancer, and use small RNAs as tools to uncover tumor-specific vulnerabilities as potential therapeutic targets. Third, we develop technologies, mainly in the areas of mammalian genetics and genomics, with a particular focus on next-generation sequencing.

We said goodbye to a number of labmates this year. Sihem Cheloufi and Ingrid Ibarra each completed their Ph.D. degrees and went on to postdoctoral fellowships at Harvard and Stanford, respectively. Elena Tonin and Elena Piccinin joined us for a brief period and then returned to Roberta Maestro's lab at the CRO, and Julia Hahn spent a time as a diploma student in the lab. Floriane Pelon, Sai Chen, and Thomas Dowling were our undergraduate summer students. Sung Wook Chi and Adam Rosebrock each left for independent positions after remarkably brief stints as postdoctoral fellows.

We were joined by Nik Rozhkov, Elena Rozhkova, Ilaria Falciatori, Simon Knott, Leah Sabin (aka Sabine), and Delphine Fagegaltier as postdoctoral fellows and Myke Malave as a computer scientist.

A selected set of laboratory projects are described in detail below.

A Genome-Wide RNAi Screen Reveals Components of the Somatic piRNA Pathway

P.M. Guzzardo, F. Muerdter

Piwi-interacting RNAs (piRNAs), together with Piwi-clade Argonaute proteins, constitute an evolutionarily conserved, germline-specific small RNA silencing system. The piRNA pathway is implicated in gene silencing, particularly of repetitive elements, as well as germline differentiation, and maintenance of germline stem cells. Accordingly, mutations in Piwi almost universally lead to sterility and germ cell loss.

In *Drosophila melanogaster*, primary piRNA biogenesis involves the processing of a primary transcript into mature small RNAs by factors that remain elusive. To search for proteins involved in biogenesis or downstream effector pathways, we performed a comprehensive genome-wide RNA interference (RNAi) screen of more than 40,000 double-stranded RNAs (dsRNAs) in a cell line derived from *Drosophila* ovaries. This cell line, the ovarian somatic sheet (OSS) cell line, expresses Piwi but not Aubergine or Argonaute3. Consequently, these cells do not show signatures of secondary piRNA biogenesis or the ping-pong cycle.

Using a quantitative polymerase chain reaction (qPCR)-based readout, we identified ~100 genes that upon knockdown lead to the derepression of a somatically expressed transposon. As part of this list, we confirm all known somatic piRNA pathway components. The newly identified genes fall into a broad range of biological pathways such as transcriptional regulators, mediators of protein–protein interactions and phospholipid biosynthesis.

By assaying several independent dsRNAs against every gene of the fly genome, this comprehensive study will provide an important foundation for understanding many aspects of piRNA biology.

A Transcriptome-Wide RNAi Screen in the *Drosophila* Ovary Reveals Novel Factors of the Germline piRNA Pathway

B. Czech, J. Preall

The *Drosophila* genome is scarred with the ravages of an ancestral battle being waged in its germline against selfish genetic elements. Transposable elements mobilize in the early stages of gametogenesis, which if left unchecked

would invade the genome and cause widespread disruption of essential genes, leading to collapse of the germline and sterility. The piRNA pathway provides a defense against selfish genetic elements by mounting a sequence-specific, RNAi-like nucleolytic surveillance mechanism. Several key factors of this pathway have been identified, but accumulating evidence has suggested that the piRNA pathway is more complex than was first appreciated, with possible ties into chromatin structure, transcriptional regulation, RNA localization, and mitotic regulation.

We have undertaken an in vivo, reverse genetic screen of unprecedented scale in order to fully describe the set of genes that participate in piRNA-mediated genomic defense. Approximately 8500 genes, spanning the entire ovarian transcriptome, were targeted by RNAi constructs driven by a germline-specific promoter. Our preliminary results have uncovered several dozen novel candidates that influence transposon levels and fertility in the female germline, including cohorts of genes belonging to shared processes such as epigenetic regulation and RNA metabolism. Ongoing analysis will serve to validate these candidates and elaborate on our understanding of their impact on the piRNA pathway, as we currently understand it.

piRNA Biogenesis: "To Cleave or Not to Cleave RNA"

A.D. Haase [in collaboration with J.J. Ipsaro (Joshua-Tor lab), Cold Spring Harbor Laboratory]

Piwi proteins and their associated small RNAs (piRNAs) act in an evolutionarily conserved genome defense pathway that silences transposons in the germline. This innate immunity system consists of both hard-wired (primary) and adaptive (secondary) components.

Despite recent progress in our understanding of piRNA biogenesis, the nucleases processing the 5′ and 3′ ends of primary piRNAs remain elusive. Recent work by our group and other investigators has implicated Zucchini (zuc) in the initiation phase of piRNA silencing. Specifically, in *zuc* mutants, primary piRNAs are depleted and piRNA cluster transcripts accumulate. This is particularly noteworthy as Zuc belongs to the phospholipase D family of phosphodiesterases, which includes phospholipases and bacterial nucleases.

Our current research focuses on detailing Zucchini's role in piRNA silencing. To this end, we are investigating the enzymatic mechanism and substrate specificity of Zucchini in vitro and characterizing Zuc-containing complexes in vivo. Taken together, these complementary approaches should establish the underpinnings of Zucchini's molecular function in primary piRNA biogenesis.

Investigating the Role of piRNAs during Germ Cell De Novo Methylation in the Mouse

A. Molaro, E. Hodges, K. Marran [in collaboration with A. Smith, University of Southern California; A. Aravin, California Institute of Technology]

During mammalian embryogenesis, germ cells undergo a genome-wide erasure and reestablishment of methylation marks. In this process of de novo methylation, PIWI-associated small RNAs (piRNAs) have been proposed to help the targeting of retrotransposons. To gain insight into this phenomenon, we performed a detailed characterization of methylation as well as transcriptional patterns at several stages of germ cell maturation in wild-type and piRNA-deficient animals (MILI nulls).

We found that despite the significant number of retrotransposons potentially targeted by piRNAs, only a small subset depends on the pathway for proper remethylation. We observed that the rest of the repeated sequences could be separated into two groups, with the vast majority being methylated and a smaller but subsequent fraction escaping remethylation, despite the presence of piRNAs. Hypomethylated repeats in wild-type and mutant animals display their lowest methylation levels over their regulatory sequences (e.g., 5′ repeats of L1 elements) and mimic some of the features of hypomethylated regions (HMRs) found elsewhere in the genome. We are now looking for the distinctive sequence features that could explain these various dynamics of de novo methylation. Our current analysis suggests that transposon copies falling in each group display different rates of conservation, closeness to consensus and transcription-factor-binding affinities. Interestingly, transcriptome analysis of the developing germ cell before, during, and after the wave of de novo methylation revealed that retrotransposons undergo a strong transcriptional reactivation prior to their silencing. By profiling small RNAs at concomitant stages, we were able to find that the selectivity of PIWI proteins for transposon piRNAs is acquired progressively as germ cells mature and anticorrelate transcript abundance. We find that whereas copies relying on piRNAs for their remethylation are heavily enriched for piRNA target sites, constitutively hypomethylated transposons lack such enrichment.

We propose that whereas most remethylation is established by default in mature germ cells, some retrotransposons mimic regions normally protected (e.g., promoters) and require the piRNA-mediated pathway to be properly targeted by de novo methylation. On the other hand, we suggest that thousands of transposons

are never remethylated and that they might constitute interesting cases of domestication and could perform a yet to be characterized regulatory function.

Structural Variation of the Genome in Breast Cancer

A. Canela

Structural variation including copy-number polymorphisms (CNVs), as well as insertions, inversions, and translocations, has an important role in tumor development. Although the mutagenic effects of translocations have long been seen as critical to the development of hematological malignancies, only recently have technological advances allowed genome-scale analysis of rearrangements in common solid tumors. We study structural variation in breast cancer cells at a single-base resolution level by next-generation sequencing. As a first approach, we focused on regions previously characterized by CGH containing CNV as landmarks for identifying complex chromosomal rearrangements. We purified these regions in breast cancer cell lines by array-based genomic capture and deciphered them by sequencing the precise structure of genomic rearrangements, which led to detection as copy-number loss and gain in the CGH (in collaboration with the Wigler lab at CSHL). Because not all the areas of CNV may contain complex chromosomal rearrangements, we used an unbiased approach with large fragment genomic libraries as "jumping libraries" to scan by sequencing the whole genome for structural variation events. We are using both approaches in a panel of breast cancer cell lines to characterize the structural variation and evaluate its role in breast tumorigenesis; this will allow us to identify driver translocations and characterize and define tumor-specific structural variation signatures. The use of genomic capture and focal resequencing for chromosomal rearrangements will give us information about differential sequence features in the breakpoints. This will further our understanding of how certain areas of the genome act as hot spots of structural variation and reveal the mechanisms behind it.

Lineage Mapping of Breast Cancer Cells during Tumor Development and Metastasis

C. Rebbeck

We have been developing a multicolor tool to investigate tumor growth and metastasis. This year, we overcame the challenges that come with using multiple recombining lox P/Cre sites and made progress on virus infection of breast tumor cells using the 4T1 cell line. With the help of a fellow lab member, we have designed programs for cell identification and color assignment, whereby various "shades" and color combinations result in a new "color" being assigned. Using a four-color construct featuring blue, green, orange, and red florescent proteins, we are able to assign a minimum of 15 colors using pictures taken with a high-powered confocal microscope. With shading, we will be able to assign more than 100 colors, allowing questions to be answered on tumor growth and metastatic seeding. The programing for cellular identification and color assignment is still being optimized, but Figure 1 shows an example of cells expressing different color combinations and the postprocessing (some processed colors/shades appear similar to the eye, but as can be seen in the original, they are different colors).

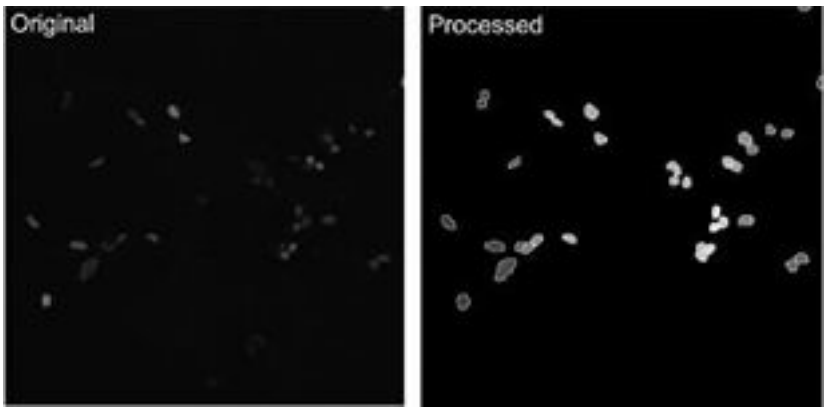

Figure 1. Example of predigital and postdigital programming to identify cells with different intensities and combinations of fluorescent color. Postprocessing assigns new colors based on a shade grouping system.

Transcriptional Profiling of Disseminated and Circulating Tumor Cells in Breast Cancer Mouse Models

R. Behrens, D. Fagegaltier, E. Wagenblast

The major cause of death for breast cancer patients is metastasis of the tumor to distant organs such as the lung, liver, and bone. Our lab is interested in identifying gene expression signatures of tumor cell populations that contribute to metastasis. For this, we have developed techniques to isolate disseminated and circulating tumor cells from breast cancer mouse models. We are using murine cell lines that were derived from a single spontaneously arising mammary tumor, which have different metastatic potentials when injected into the mammary gland of mice. These murine cell lines are tagged with a green fluorescent protein (GFP) fluorophore for identification purposes. Circulating tumor cells are isolated from the blood using a combination of immunomagentic separation and fluorescence-activated cell sorting (FACS). Disseminated tumor cells from various metastatic organs are isolated by FACS and laser capture microscopy. Upon isolation, gene expression profiles are characterized using next-generation sequencing. We are comparing these gene expression signatures to other less metastatic cells in order to identify genes that are responsible for metastasis formation. Subsequently, we will be validating potential metastatic regulators by conducting knockdown and overexpression studies in vitro and in vivo.

Genomic and Epigenetic Changes Occurring during Carcinogenesis: A Fly Perspective

D. Fagegaltier, E. Wagenblast

Other questions related to carcinogenesis that remain largely unexplored are how cells become transformed, which pathways are involved in reaching a transformed state, and whether each cell takes the same route to reach such a state. To this end, we are using the *Drosophila* model to study the basic mechanisms by which genomes coordinate their genetic and epigenetic responses toward a transformed state activated by specific oncogenes or tumor suppressors. This STARR consortium project has three major aims: (1) to generate *Drosophila* cell lines with cancer-relevant genotypes, (2) to profile the transcriptional and epigenetic changes that occur during the establishment of these cell lines, and (3) to address whether cells remain addicted to the presence of the initiating oncogene or loss of tumor suppressor and to establish which factors are required for the cells to proliferate and maintain a transformed state. We have established various cell lines expressing an oncogene or depleted for a tumor suppressor. A pilot array experiment on primary cell lines derived from *ras*-oncogene-expressing embryos suggests that cells undergo major epigenetic changes via the Polycomb group of proteins before reaching a transformed state. To further confirm the role of these proteins during transformation, we are comparing the transcriptomes of a larger set of transformed cell lines using next-generation sequencing. By dissecting the progressive transcriptional changes generated during transformation, these studies will shed light on the general mechanisms and pathways leading to tumorigenesis and reveal changes specific to each oncogenic molecule studied.

Patterns of Aberrant DNA Methylation in Cancer

F. Rollins

During the past year, I have been working on understanding the patterns of DNA methylation in non-small-cell lung cancer. We have been using cancer cell lines, and through the combination of bisulfite conversion and high-throughput whole-genome sequencing, we are unraveling the epigenetic patterns of cancer cells. We have identified a set of characteristics that not only are specific to cancer cells, but could also underlie some of the most basic phenotypes of cancer cells, including increased rates of mutation and genomic instability. These insights give us a further understanding of how cancer cells are different from normal cells and will provide a foundation for future cancer research.

Role of an X-linked miRNA Cluster in Mouse Spermatogenesis

I. Falciatori [in collaboration with S. Raffii, Cornell University]

Spermatogonial stem cells (SSCs) are the stem cells responsible for the production of sperm in mammals. When cultured in vitro, they can spontaneously give rise to a different kind of cells. These cells are called mammary gland stem cells (MaSCs) and they resemble embryonic stem cells in terms of both phenotype and developmental potential. In an attempt to characterize the difference in the small RNA profile between SSCs and MaSCs, we identified an X-linked cluster of microRNAs (miRNAs) that is

highly expressed in SSCs and is significantly down-regulated after transition to MaSCs. We established that this cluster is specifically expressed in testis in several stages of development, but it is nondetectable in other organs. We therefore became interested in determining the function of this miRNA cluster during spermatogenesis. To this end, we decided to ablate this cluster during spermatogenesis in mice and analyze their phenotype. We prepared a modified bacterial artificial chromosome (BAC) in which the miR cluster is flanked by LoxP sites. This will allow us to generate mice in which this cluster could be selectively ablated at different stages of spermatogenesis. We will then assess spermatogenesis in these ablated mice looking for defects that will allow us to infer the function of the cluster. Because miRNAs exert their function by down-regulating their target genes, we are going to overexpress the entire cluster in embryonic stem cells and look for transcripts that become down-regulated. To achieve this goal, we have produced a modified BAC in which we added a TRE promoter to drive the expression of the cluster in embryonic stem cells under the control of tetracycline. We are in the process of producing the transgenic embryonic stem cell lines carrying this BAC. We will then compare their mRNA profiles in the presence or absence of tetracycline to find potential miRNA targets. These targets will then be validated in SSCs.

Studies of piRNA Biology in Mice and Flies

K. Wasik, V. Vagin

Preliminary evidence suggests that meiosis in RNF17 knockout spermatocytes is disrupted, resulting in fewer haploid cells in the testes and the absence of elongating spermatids. Comparison of the small RNAs between wild-type and RNF17 knockout mice revealed an ~10-fold reduction in pachytene piRNAs in RNF17 mutants. However, the expression levels of pachytene piRNA precursor transcripts were unaffected. Immunofluorescence of wild-type testes showed partial colocalization of RNF17 with Miwi in pachytene spermatocytes, but not in round spermatids. In contrast, in round spermatids, Miwi is present in the chromatoid body and RNF17 localizes in uncharacterized granules. Strikingly, in RNF17 KO mice, Miwi does not localize to chromatoid bodies. We hypothesize that RNF17 participates in the loading of pachytene piRNAs onto Miwi. This loading happens either in RNF17 granules or in chromatoid bodies. We are currently analyzing the RNA and protein content of chromatoid bodies and RNF17 granules.

We have also been searching for new piRNA biogenesis factors in flies. Because of the significant difficulties in mapping the Dippp mutation, we decided to map another mutation—Z20529, a Zucker collection mutant. The Z20529(–/–) fly is defective in primary piRNA biogenesis in germline and somatic cells. We have used a recombination mapping strategy based on the same principles as for Dippp. The result of this recombination mapping pointed to a 3-Mb region on chromosome 2R. We have started crosses with flies harboring deletions in the region of interest. So far, 10 of 11 deletion lines have been negative for the mutation. In addition, we have also crossed the Z20529 fly with other mutants in the piRNA pathway on chromosome 2. All of these mutations complemented Z20529. In our fly mutant collection, however, two other mutants (Z20149 and Z26032) did not complement the Z20529 mutation. We expected these three fly lines to have a mutation in the same gene. Hence, we decided to perform genomic sequencing on all three mutants and use single-nucleotide polymorphism (SNP) calling to find a gene that is mutated in all three fly lines. The SNP calling was performed in collaboration with Michael Shatz. We are currently filtering out our candidates. Additionally, we performed small RNA cloning and prepared transcriptome libraries from Z20529 heterozygous and homozygous flies to better characterize the phenotype.

Role of Long Noncoding RNAs in Normal Hematopoiesis and Malignant Transformation

L. Sabin

Precise control of gene expression is achieved through multiple layers of regulation that influence the transcriptional and epigenetic states of genomic loci. Long noncoding RNAs (lncRNAs) have recently emerged as a novel class of molecules with regulatory potential. However, ascribing clear functional and biological roles to these noncoding transcripts has been challenging. Although the function of most lncRNAs remains unknown, many of the lncRNAs that have been characterized have roles in transcriptional and epigenetic regulation of gene expression by acting as molecular scaffolds to recruit epigenetic modifiers and other protein complexes to target genomic loci.

One developmental process that requires coordinated regulation of gene expression is hematopoiesis. Importantly, the malignant transformation of various hematopoietic lineages leads to the development of several forms of leukemia and lymphoma, and the disruption or misregulation of epigenetic modifications is a common fea-

ture of cancer cells. Therefore, we are studying the function of lncRNAs in the murine hematopoietic system, with the aim of identifying lncRNAs that have critical roles in the epigenetic control of gene regulation. In particular, we hope to uncover lncRNAs responsible for regulating the genes involved in promoting or suppressing tumorigenesis.

To this end, we plan to select a set of candidate lncRNAs for further studies using an integrated data set that includes RNA-Seq and chromatin immunoprecipitation (ChIP)-Seq data from normal and transformed cells. We are using high-throughput sequencing to profile lncRNA expression in various cell types within the hematopoietic lineage, including progenitor populations and mature cell types from both myeloid and lymphoid lineages. We also hope to profile lncRNA expression in two mouse models of blood cancer: the MLL-AF9 model of acute myeloid leukemia and the Eμ-*myc* model of B-cell lymphoma. We then plan to use short hairpin RNA (shRNA) knockdown technology to determine which candidate lncRNAs have important biological roles in vivo. We will deplete lncRNA candidates from hematopoietic stem cells, and use the lncRNA-deficient cells in bone marrow reconstitution assays to test whether candidates are involved in self-renewal, differentiation, or malignant transformation. We hope that this work will provide important insights into lncRNA function in the epigenetic control of gene expression during normal hematopoiesis and malignant transformation.

miRNA Target Sites in Mouse Embryonic Stem Cells

M. Kudla

miRNAs together with Argonaute proteins form a ribonucleoprotein complex involved in posttranscriptional silencing of transcripts. Although Argonaute is the functional partner in this complex, miRNAs are the specificity factor, determining which transcripts will be silenced. The foundation of this target recognition mechanism is sequence complementarity between the miRNA and part of the mRNA. In plants, the level of complementarity is high enough to make a bioinformatic prediction of the target sites a straightforward task. In animals in general, but particularly in mammals, the complementarity requirement is relaxed to such an extent that similar predictions are very inaccurate. An experimental method of discovery and validation of target sites is required. We have been using the HITS-CLIP (high-throughput sequencing isolated by cross-linking immunoprecipitation) method for characterization of mouse embryonic stem cells and cells derived from them through targeted differentiation. We are now able to obtain a map of target sites used by miRNA:Argonaute complexes on inhibited transcripts. Using statistical techniques, based on negative binomial distribution, we are also able to identify target sites that change systematically upon transition from one condition to another.

The Origins and Functions of piRNAs in *Caenorhabditis elegans*

W.S. Goh

piRNAs are germline-specific small RNAs that load onto piwi proteins, and their loss leads to sterility in all piRNA-possessing species. In light of piRNAs' dicer independence, genomic cluster arrangement, and strand bias, a long single-stranded piRNA precursor is hypothesized to be the intermediate molecule in piRNA biogenesis. However, a concrete piRNA biogenesis model has yet to be established in any species.

The nematode *C. elegans* is a well-established model organism for performing genetic screens. *C. elegans* also possess piRNAs, which are called 21Us, that load onto the piwi protein PRG-1. However, much less is known about piRNAs in *C. elegans* than in other species. To elucidate piRNA biogenesis in *C. elegans*, I performed a genome-wide RNAi screen to identify novel 21U factors that alter 21U levels as measured by quantitative real-time PCR: ~25 suppressors and ~50 biogenesis factors were identified, where the strongest characterized factor is ulp-5, a desumoylating factor. This factor is involved in the sumo pathway, which among other processes, is known to regulate protein degradation by the ubiquitin pathway. Coincidentally, other members of the sumo pathway were identified in orthogonal screens for piRNA factors in fly ovarian sheet cell lines and whole flies. Future work on this screen will focus on characterizing ulp-5 and the other hits identified in this screen.

An EMS Screen Revealed a Functional Link between Phosphatidic Acid and Protein Phosphatase 1 in the Primary piRNA Pathway

V.V. Vagin, Y. Yang, E. Harrison

In flies, the piRNA pathway silences transposons in the germline and somatic follicular cells of ovaries. Despite recent progress in understanding piRNA biogenesis,

there is still little known about primary piRNA processing. To elucidate this mechanism, we conducted an ethylmethane sulfonate (EMS) screen to identify new primary piRNA components.

We found that the mitochondrial glycerol-3-phosphate *O*-acyltransferase gene (*CG5508*) was a primary piRNA gene. In the CG5508 mutant, PIWI delocalizes from nucleus to Yb body, and the elimination of mature primary piRNAs correlates with accumulation of piRNA precursor transcripts. Overall, the mutant phenotype is similar to mutations in known primary piRNA genes.

We genetically dissected mitochondrial phospholipid biogenesis to understand its role in the primary piRNA pathway. Knocking down of phospholipid genes indicates that phosphatidic acid (PA) is essential for primary piRNA production. Interestingly, it was shown that PA regulates activity of protein phosphatase 1 (PP1). RNAi against PP1 subunits releases transposon silencing.

Scanning the Genome for Potent shRNAs

X. Zhou

The goal of my work is to improve RNAi tools. miRNA-based shRNA has become a versatile and powerful RNAi tool that enables stable and tightly regulated expression of RNAi triggers of any sequence. However, shRNAs have some limitations. Among all possible sequences that target a given RNA transcript, very few are efficient. Due to limited knowledge of miRNA biogenesis and target interaction, identification of potent RNAi triggers remains difficult. I aim to develop a deep-sequencing-based high-throughput "molecular sensor assay" that measures and quantifies shRNA potency by measuring target RNA cleavage efficiency. The pilot experiment using previously characterized shRNAs showed a correlation between cleavage efficiency and shRNA potency. This method is being tested on a larger scale. If successful, we will be able to identify potent shRNAs from a large number of candidates quickly and quantitatively. Moreover, the quantitative feature will lead to better understanding of shRNA biogenesis and target interaction.

Proteomic Approaches to piRNAs

Y. Yu

I have optimized several systems to perform immunoprecipitation-SILAC (stable isotope labeling by amino acids in cell culture) experiments to identify piwi-interacting proteins. With help from Vasily Vagin (CSHL), I have achieved high labeling efficiencies for various samples including flies and mouse embryonic testis. I am actively collecting labeled samples from transgenic animals, and immunoprecipitation-SILAC will be performed once they are ready. Through piwi/SILAC from OSS (ovary somatic sheet) cells, I have identified ~190 piwi-interacting candidates. Known partners such as Yb, Armitage are among the top hits, and some of the new targets have been verified through RNAi (e.g., ctp). Although many targets remain interesting, systematic cross-comparison of the proteomic data with results of RNAi screens for new piRNA factors has identified a number of candidates important for the piRNA pathway. Notably, eight of the overlapping candidates are known to affect sterility. The plan is to see which of those candidates affect piRNA processing or effector steps through small RNA cloning.

To identify piRNA as the 3′-end processing factor (the "trimmer"), I have recently generated stable silkworm cell lines expressing Halo-tagged proteins such as siwi, BmAgo3, and dHen1 in the hope of pulling down relevant nucleases. By taking advantage of the covalent binding between the Halo tag and its specific chloro-alkane ligand, I was able to cross-link protein partners of Halo fusion proteins and at the same time wash away any contaminants under denaturing conditions. Currently, I am optimizing the cross-linking and purification conditions.

Desiging Potent and Specific Tools to Find New Anticancer Targets

S. Knott

Functional screens, aimed at identifying putative therapeutic agents, have become a powerful weapon in the "war on cancer." Our improved understanding of the biological parameters that determine the efficacy with which shRNA molecules suppress their intended target genes is a fundamental reason for this technology's movement to the forefront of cancer research. Recently, the Hannon lab, in collaboration with the Lowe lab (both at CSHL), has developed a high-throughput method for identifying what sequence-based parameters drive shRNA efficacy. Using the results of these methods, we have developed a computational tool aimed at predicting the optimal shRNA sequence for any given gene target. Armed with this tool, we have predicted optimal genome-wide shRNA libraries for the human, mouse, and rat genomes. These libraries are currently under construction and should be available to the scientific community in the summer of 2012.

Argonautes in Gene Regulation

F. Karginov

Argonaute proteins are guided by their miRNA partners to mRNA targets, leading to regulation of the mRNA in a spatially and temporally controlled manner. In recent years, it has become more evident that the interactions between Ago/miRNAs and mRNAs are further affected by ancillary RNA-binding proteins, creating a combinatory system of regulation akin to transcription factors. We are using novel transcriptome-wide technologies (CLIP) to identify the sites of interaction between Argonaute and mRNAs, to dissect the Ago regulatory networks and their integration with other RNA-binding proteins. More specifically, I have looked at the changes in Argonaute occupancy as a result of acute cellular stress to assess the participation of Ago in this process. We have identified global changes in the interactions of Ago with their mRNA targets, as well as transcript-specific cases of Ago regulation, that may help the cell adapt to the stress condition.

Genetics Approaches to Anticancer Therapy

K. Chang, S. Knott, S. Shaw, S. Fenoglio, A. Valentine, K. Marran, C. Peterson, Y.J. Yang

The principal goal of our group is to build tools to harness RNAi to perform large-scale loss-of-function studies in mammals. One of our aims involves a continued effort to develop and construct shRNA libraries capable of silencing every gene in the human and mouse genomes. To date, we have completed construction for our third-generation mammalian shRNA libraries, and these new reagents have been made available to the research community as with our previous versions. Each generation of the shRNA library has been designed based on our growing knowledge of the biochemical and molecular basis of RNAi. The most significant advance of the third-generation RNAi resources is the functional validation of efficacy for each individual shRNA design in these libraries. This huge undertaking was made possible using a highly parallel, large-scale "sensor" technology we have developed in collaboration with Dr. Scott Lowe's lab (of CSHL and Memorial Sloan-Kettering Cancer Center) to validate pools of 22,000 shRNAs at a time. In total, the final human and mouse third-version libraries each comprise 80,000 sequence-verified shRNAs (four shRNAs per gene). Due to the considerable undertaking to validate all the shRNAs, we have recently developed a new technology using a "molecular sensor" approach that could measure efficacies of shRNAs in a fraction of the time (this is further elaborated in another section of our lab's annual report). Furthermore, the algorithm (DSIR) used to predict shRNA designs for the third-generation libraries produced more potent shRNAs for each gene than any of our previous shRNA collections.

Large-scale in vitro RNAi screening in mammalian cells was developed by our lab in 2008, and since that time it has become a widely used tool to uncover genetic dependencies, particularly in cancer cells. The target genes or the molecular pathways that define the dependencies represent essential processes or modifiers of drug resistance/sensitivity of tumor cells that can be explored for therapeutic benefit. To facilitate high-throughput RNAi screens in cell culture, we have optimized and industrialized our original screening methodology for genome-wide interrogation. During the past 2 years, we have been part of the target discovery group for the Stand-Up to Cancer (SU2C) effort to combat breast cancer. This is a very comprehensive and rigorous study of all subtypes of breast cancer using state-of the-art research tools and expertise from many accomplished investigators to yield new clinical impacts within a relatively short period of time. Our overall objective for SU2C is to apply genome-wide RNAi screening, using our third-generation shRNA library, to uncover genetic vulnerabilities and pathways that can modify currently used targeted therapies (tamoxifen, frastuzumab, and lapatinib) in de novo and acquired resistance. To date, we have completed 26 genome-wide screens in tumor-derived cell lines representing all three treatment classes of breast cancer (ER-positive, Her2-positive, and triple-negative disease). Another study, ongoing for more than a year and also involving in vitro genome-wide RNAi screening, is through funding from the Lustgarten Foundation to find targeted therapeutic approaches against pancreatic cancer. We have begun screening a panel of 14 pancreatic cancer cell lines to uncover genetic vulnerabilities and to probe sensitizing targets against gemcitabine (chemotherapy) for new combinational therapies to treat this highly aggressive disease. To facilitate our large-scale screening studies, we have now established a core screening group in our lab to undertake these projects.

Our third objective is to develop new tools for RNAi screens in animals. Our approach to in vivo RNAi screens is based not only on the need to overcome the biological limitations of cell culture, but also on our intent to directly interrogate human cancer cells that have not been adapted in vitro. To this end, we will screen cells

from human primary tumor-derived xenograft models (PDX) in immunocompromised mice (subcutaneous and orthotopic sites). Protocols and tools are currently being developed. This involves testing an array of promoters for optimal expression of shRNAs in vivo. In addition, we have developed a new, regulatable expression vector system to facilitate in vivo screening. Our initial goal is to screen pancreatic cancer PDX models (Dr. Eshelman's lab of Johns Hopkins University) as part of the Lustgarten project and also breast cancer PDX models (Dr. Alana Welm of University of Utah). Ultimately, we wish to expand the application of this new in vivo screening technology for other cancers.

During the past year, we have began collaborating with Dr. Charles Sawyers' lab (Memorial Sloan-Kettering Cancer Center) to apply RNAi screening to understand the genetic alterations that promote tumor progression and therapy resistance to castration-resistant prostate cancer (CRPC) (funded by the STARR Cancer Consortium). A considerable difficulty is that castration-resistance phenotypes can only be effectively measured in vivo, necessitating the use of in vivo RNAi. Screening paradigms will be carried out in two models: the mouse Myc-CaP prostate cancer xenograft and the LNCaP/AR human prostate xenograft.

Forays into Neuroscience and Beyond

D. Bressan

My research focuses on the development of new molecular techniques to investigate connectivity and gene expression in the brain.

My main project is to develop a system to convert the complexity of the synaptic connections in the brain into a "genetic" complexity, by appending unique "bar codes" to every neuron and causing them to fuse at the synapse, forming a pair that "freezes" the identity of the connection in a conformation that can be purified and sequenced using the instruments of genomics.

Because we can now routinely sequence more than 1 billion bar codes in ~2 weeks in a single run of a sequencing machine, this technique could allow resolving an entire connectome (the full map of connections in a brain) in a really fast and affordable way. We are currently testing and troubleshooting the protocol in the worm *C. elegans*, the only animal for which a reference connectome exists.

My second project is to develop a way of labeling and subsequently purifying the entire translatome (the collection of mRNAs being translated by ribosomes) of a cell or subcellular compartment with great spatial accuracy, by means of a photoactivable "tag" that binds to ribosomes in vivo only upon irradiation by a laser.

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Greg Hannon (*seated, far right*) and lab members

INTEGRATED APPROACHES TO CANCER GENETICS AND BIOLOGY

S. Lowe I. Appelmann, J. Bolden, J. Cappellani, C. Chen, A. Chicas, L. Dow, S. Ebbesen, C. Fellmann, D. Grace, X. Guo, C.-H. Huang, T. Kitzing, Y. Liu, A. Lujambio, B. Ma, K. McJunkin, O. Mert, C. Miething, Y. Park, P. Premsrirut, A. Rappaport, A. Saborowski, M. Saborowski, C. Scuoppo, A. Shroff, J. Simon, M. Spector, V. Sridhar, N. Tasdemir, V. Thapar, S. Weissmueller, J. White, Z. Zhao, J. Zuber

Cancer arises through an evolutionary process whereby normal cells acquire mutations that erode growth controls, leading to the expansion of aberrantly proliferating cells. Such mutations activate oncogenes or inactivate tumor suppressors, each bestowing new capabilities on developing cancer cells. Our research is based on the premise that the path of cancer evolution dictates a tumor's subsequent response to therapy and creates unique vulnerabilities that represent therapeutic opportunities. Hence, we apply mouse models, RNA interference (RNAi), and cancer genomics in a coordinated effort to identify components of tumor suppressor gene networks and to understand molecular determinants of treatment response. We also use conditional RNAi technology to identify tumor maintenance genes and explore the cell intrinsic and extrinsic mechanisms involved in tumor regression. Our goal is to gain a more comprehensive understanding of tumor suppressor networks and identify cancer maintenance genes that will be useful therapeutic targets relevant to specific cancer genotypes.

Accelerating the Pace of Discovery: Mosaic Mouse Models and RNAi

J. Bolden, J. Cappellani, C. Chen, L. Dow, S. Ebbesen, C. Fellmann, D. Grace, X. Guo, Y. Liu, K. McJunkin, C. Miething, Y. Park, P. Premsrirut, A. Saborowski, M. Saborowski, J. Simon, V. Sridhar, V. Thapar, J. White [in collaboration with G. Hannon, C. Malone, R. Sordella, M. Comilio, S. Kim, Cold Sring Harbor Laboratory; S. Elledge, Harvard Medical School; R. Dickins, Walter and Eliza Hall Institute]

Genetically engineered mice provide powerful tools to study cancer, yet their generation and analysis is time-consuming and expensive. Loss-of-function studies—essential for characterizing tumor suppressor genes or candidate drug targets—are laborious and typically require tissue-specific conditional knockouts harboring multiple alleles. Moreover, to validate drug targets, genes must be inactivated in each cell of an established tumor, a technical challenge that has only been achieved in a few instances. Even where feasible, conditional gene deletions are not reversible and hence do not mimic transient pharmacologic target inhibition. Consequently, traditional mouse-modeling approaches are not sufficiently fast or flexible to comprehensively model cancer heterogeneity or evaluate the range of emerging drug targets (Dow and Lowe 2012).

To address these issues, we developed strategies to accelerate genetic studies in mice. On the one hand, we have taken a "mosaic" approach, where cancer-predisposing alleles or reporters are transduced into stem and progenitor cells derived from different tissues or mouse strains; these modified cells are then transplanted orthotopically into syngeneic recipients. Because the genetic configuration of the target cells, transduced genes, and recipients is easily varied, genotypic diversity can be readily produced. On the other hand, we have created a "speedy mouse" platform, in which murine embryonic stem cells (ES cells) can be derived from mice harboring several cancer-predisposing alleles, enabling the subsequent production of large numbers of mice by blastocyst injection or tetraploid embryo complementation without extensive intercrossing of germline strains (Premsrirut et al. 2011; Dow et al. 2012). To date, we have developed mosaic models of lymphoma and leukemia (Zuber et al. 2011), as well as carcinomas of the liver, breast, bile duct, and pancreas. In addition, we have produced speedy mouse models of lung adenocarcinoma (Premsrirut et al. 2011), with efforts aimed at producing models of melanoma and carcinomas of the colon, breast, liver, and ovary in progress. Armed with these models, a single investigator can study the impact of many cancer genotypes on tumor behavior and rapidly test new hypotheses based on their results.

To facilitate loss-of-gene-function studies, we worked with Gregory Hannon at CSHL to develop inducible short hairpin RNA (shRNA) technology as a rapid and

reversible alternative to conditional gene deletions. This platform is based on the fact that shRNAs embedded in a natural microRNA (miRNA) structure are effectively expressed from polymerase II promoters, thereby enabling robust gene knockdown using constitutive and inducible vectors. During the last year, several convergent efforts came to fruition, enabling the development of better vectors and screening methods that should increase the efficiency and output of negative selection shRNA screens performed for the purpose of identifying cancer drug targets (Zuber et al. 2011). In addition, a "sensor" assay was developed to sort through thousands of shRNA sequences to identify the most potent. In addition to describing a general strategy for the production of large-scale validated shRNA libraries, the data yielded by these analyses produced new information related to the underlying biology of efficient RNAi (Fellmann et al. 2011).

We also developed a scalable platform for producing mice harboring inducible shRNAs with the long-term goal of producing a general platform that will enable the spatial, temporal, and reversible suppression of any mammalian gene. The system integrates our advances on shRNA vector design (inducibility via a tet-transactivation system, enhanced potency, and coupling shRNA expression to a fluorescent reporter) with a recombination-mediated cassette exchange approach developed in the Jaenisch lab that enables rapid and reproducible targeting of the inducible shRNA cassette to a defined genomic locus in ES cells (McJunkin et al. 2011; Premsrirut et al. 2011). Mice can then be produced rapidly using tetraploid embryo complementation. Currently, our laboratory has produced more than 1000 ES cell lines and 75 mouse strains and is using the resulting cells and mice to study tissue-specific and reversible suppression of tumor suppressors, miRNAs, drug targets, and essential genes.

To accelerate the pace at which we can determine how particular genes modulate disease phenotypes, we developed "speedy mouse" technology in which ES cells are rederived from mice harboring a recombination-mediated cassette exchange homing locus with certain cancer-predisposing alleles (Premsrirut et al. 2011; Dow et al. 2012). With this approach, shRNAs targeting candidate disease modifiers (new cancer genes, or genes involved in tumor maintenance) can be introduced into these ES cells, enabling researchers to study the impact of studying many genes on the progression or regression of disease simply by controlling the time and length of gene inhibition by doxycycline addition. Currently, we are building such platforms for a variety of cancer genotypes and anticipate that the use of these systems will substantially impact our research in the years to come.

Cell Survival and Cell Death

Y. Liu, C. Miething, C. Scuoppo, J. Simon [in collaboration with J. Reyes, A. Kraznitz, C. Ruse, D. Pappin, Cold Spring Harbor Laboratory; J. Pelletier and L. Linqvist, McGill University]

Since its inception, our laboratory has studied genes that modulate cell death and survival. Current work in this area exploits the Eμ-myc B-cell lymphoma model, where disruption of the ARF-p53 pathway, or overexpression of the prosurvival genes *Bcl-2* or *Akt*, cooperates to accelerate lymphomagenesis. Following up "hits" from in vivo RNAi screens (see below), we are characterizing new tumor suppressors that modulate apoptosis. This year, we identified two tumor suppressors involved in polyamine biology: Amd1, an enzyme that catalyzes the rate-limiting step in the synthesis of spermidine, and eIF5A, the only protein to incorporate hypusine, a modified amino acid derived from spermidine. Remarkably, this pathway is conserved from archaebacteria to humans, involving two enzymes (DHPS, DOHH) that specifically target the same highly conserved lysine on—and apparently only on—eIF5A. Indeed, a secondary RNAi screen identified two other enzymes (SRM, DHPS) needed for the production of hypusine as tumor suppressors, further emphasizing the relevance of the hypusination pathway.

Although the biochemical action of eIF5A in cancer is not established, our studies suggest that hypusination of eIF5A potentiates apoptosis through p53-independent control of Bax, a proapoptotic member of the Bcl-2 family known to function as a tumor suppressor in this system. As a consequence, our study identified a previously unanticipated tumor suppressor network that acts by suppressing apoptosis. Moving forward, we will continue to explore the underlying biological mechanism of the new tumor suppressor network we identified, as well as characterize other tumor suppressors that were identified in our screen. Using the inducible shRNA transgenic mice described above, we are also studying how control of cell survival by eIF4E and PTEN impact tumor initiation and maintenance.

Senescence and Self-Renewal

J. Bolden, A. Chicas, O. Mert, C. Scuoppo, J. Simon, N. Tasdemir [in collaboration with E. Bernstein and A. Kapoor, Mt. Sinai School of Medicine; V. Krizhanovsky and A. Sagiv, Weizmann Institute; X. Wang, Tsinghua University]

Cellular senescence is a stable form of proliferative arrest that acts as a potent barrier to cancer development

and may contribute to various age-related pathologies. Initially defined by the phenotype of cultured fibroblasts undergoing replicative exhaustion, senescence can be triggered in many cell types by a range of cellular stresses including the aberrant expression of mitogenic oncogenes. The senescence program is controlled by an interplay between the retinoblastoma (RB) and p53 tumor suppressor networks, and senescent cells typically display a characteristic transcriptional profile involving down-regulation of growth-promoting genes, up-regulation of cell cycle inhibitors, and changes in the expression of secreted molecules involved in extracellular matrix production and immune surveillance.

We continue to search for other pathway components that may contribute to senescence. We previously showed that RB but not p107 or p130 is required for the establishment of cellular senescence and proposed that this nonredundant function of RB is one mechanism by which RB exerts its tumor suppressor function. To explore the mechanistic basis for this effect, we took a nonbiased approach to identify changes in histone modification patterns occurring in senescent cells. Our work showed that RB mediates a senescence-specific loss of H3K4 methylation on certain E2F target genes by binding the H3K4 demethylases Jarid1a and Jarid1b. In a parallel effort, we used gene expression profiling to identify new senescence regulators, and in doing so, we identified a poorly characterized E2F family member as a novel link between the p53 and RB pathways.

In addition to the stable arrest phenotype controlled by RB and p53, senescent cells display a "senescence-associated secretory phenotype" (SASP) involving the production of factors that reinforce the senescence arrest, alter the microenvironment, and trigger immune surveillance of the senescent cells. Through a proteomics analysis of senescent chromatin, we identified the nuclear factor-κB (NF-κB) subunit p65 as a major transcription factor that accumulates on chromatin of senescent cells (Chien et al. 2011). We found that NF-κB acts as a master regulator of the SASP, influencing the expression of more genes than RB and p53 combined. In cultured fibroblasts, disabling the SASP by NF-κB suppression causes escape from immune recognition by natural killer (NK) cells and cooperates with p53 inactivation to bypass senescence and, in a mouse lymphoma model, bypasses treatment-induced senescence producing drug resistance, early relapse, and reduced survival. Our results demonstrate that NF-κB-mediated control of the SASP influences both cell-autonomous and non-cell-autonomous aspects of the senescence program and identify a tumor suppressive function of NF-κB that contributes to the outcome of cancer therapy.

We have also studied mechanisms of immune surveillance of senescent cells. For example, during liver fibrosis, hepatic stellate cells initially proliferate and secrete extracellular matrix components that produce the characteristic patterns of fibrosis; however, they eventually undergo senescence and are cleared by NK cells and other components of the innate immune system. This year, we studied how NK cells target senescent cells and examined the impact of disrupting this process on liver fibrosis in vivo. We showed that the granule exocytosis pathway, and not the death receptor pathway, mediates killing of the senescent cells by the NK cells, and without this pathway, mice are more prone to fibrosis (Sagiv et al., in press). This pathway bias is determined by DcR5, a decoy receptor that attenuates signaling through the death receptor pathway and is up-regulated in senescent cells. Currently, we are investigating how the disruption of senescence in stellate cells ultimately influences the development of liver cirrhosis and carcinoma development. Early data suggest that the program may suppress the development of these conditions in a non-cell-autonomous way.

Identifying Tumor Maintenance Genes and Therapeutic Targets

I. Appelmann, J. Cappellani, C. Chen, C.-H. Huang, Y. Liu, A. Lujambio, B. Ma, A. Rappaport, A. Saborowski, A. Shroff, J. Simon, N. Tasdemir, V. Thapar, S. Weissmueller, J. Zuber
[in collaboration with G. Hannon, C. Vakoc, J. Shi, E. Wang, Cold Spring Harbor Laboratory; S. Kogan, University of California, San Francisco; J. Bradner, Dana-Farber Cancer Institute]

We are also using RNAi to systematically identify genotype-specific drug targets. Although many investigators focus on human-derived cancer cell lines for these studies, our efforts heavily use murine model systems because of the defined nature of the driving genetic events and our ability to rapidly extend results to in vivo systems. By combining inducible RNAi systems and linked fluorescent reporters, many candidate tumor maintenance genes can be identified and rapidly triaged to focus on those that show the most promise. Although our efforts to combine technologies for target discovery focus on a variety of cancers, our initial successes have come largely from the study of hematopoietic malignancies.

One area of investigation focuses on the identification of tumor maintenance genes in mouse models of refractory AML. Previous work from our laboratory has

developed such models and shown that, for example, those triggered by mixed lineage leukemia (MLL) fusion oncoproteins are particularly refractory to conventional cancer therapy. By characterizing how MLL fusion proteins promote self-renewal in a mouse model of acute myeloid leukemia (AML), we identified the Myb transcription factor as specifically required for the maintenance of an aberrant self-renewal program triggered by MLL-fusion proteins and found that inhibition of Myb at an advanced disease stage could eradicate leukemia in mice (Zuber et al. 2011). These observations provide direct evidence that targeting aberrant self-renewal programs would provide a therapeutic benefit. With Christopher Vakoc at CSHL, we screened an shRNA library targeting "epigenetic" regulators and have identified several histone-modifying activities whose suppression causes the selective arrest or death of leukemic cells. One of these, Brd4, is the target of an available small-molecule inhibitor that we showed is active against our AML models, certain human AML lines, and primary cancer material (Zuber et al. 2011).

Interestingly, gene expression changes generated following genetic or pharmacologic inhibition of Brd4 are remarkably similar to those observed upon suppressing MLL-AF9 or Myb, suggesting that MLL-AF9, Myb, and Brd4 functionally intersect within a common transcriptional circuit essential for malignant self-renewal. A key effector of this program is the oncoprotein Myc, which has been validated as an attractive therapeutic target but has to date escaped efforts at pharmacological inhibition. Although the precise mechanism remains to be further defined, targeting Brd4 extinguishes Myc expression and limits self-renewal with selectivity for the leukemic context, thus averting hematopoietic toxicities potentially associated with systemic Myc inhibition. Thus, our studies identify Brd4 as a promising therapeutic target in AML and identify a small molecule that efficiently targets Myc.

We will continue to apply our mouse modeling and RNAi technologies to identify context-dependent tumor maintenance genes, particularly in those cancers that are refractory to current therapies. Accordingly, we continue to work with Chris Vakoc to characterize the PRC complex as a therapeutic target in some types of AML (Shi et al., in press) and are producing optimized shRNA libraries targeting relevant drug targets and developing screening strategies in vivo. Beyond mosaic models, our shRNA transgenic platform provides a rapid way to validate targets and elucidate the potential toxic effects of transient target inhibition. For example, we showed that INK4a/ARF reactivation in aggressive lung carcinomas leads to tumor regression associated with enhanced apoptosis (Premsrirut et al. 2011). We also showed that Apc loss is required to maintain the proliferation of T-cell acute lymphoid leukemia, thus validating the Wnt pathway as a therapeutic target (Premsrirut et al. 2011). Current efforts are studying the impact of PTEN restoration in aggressive T-cell lymphomas. Finally, as a proof of principle, we showed that inhibiting DNA synthesis in vivo through suppression of replication protein A could produce toxic but reversible effects in mice.

Integrated Approaches to Cancer Gene Discovery

C. Chen, X. Guo, T. Kitzing, Y. Liu, A. Lujambio, B. Ma, C. Miething, M. Saborowski, C. Scuoppo, A. Shroff, J. Simon, M. Spector, V. Thapar, S. Weissmueller, Z. Zhao [in collaboration with S. Roessler and X. Wang, National Institutes of Health; A. Kraznitz, I. Issifov, M. Wigler, J. Hicks, G. Hannon, G. Atwal, S. Powers, Cold Spring Harbor Laboratory; S. Allen, North Shore/Long Island Jewish Hospital]

Cancer genomes are complex and can harbor cancer-promoting "driver" mutations together with "passenger" mutations that have no biological effect. To accelerate cancer gene discovery, we use mosaic mouse models to filter candidates obtained through genomics to identify genes whose activity contributes to tumorigenesis. We presume that (1) recurrent amplifications and deletions in human tumors are enriched for oncogenes and tumor suppressors, respectively, and (2) lesions that give rise to cancer in humans often do so in mice. This approach has led to the identification and validation of more than 50 oncogenes and tumor suppressor genes during the last several years.

We developed strategies to multiplex this approach by transducing tissue stem and progenitor cells with pools of cDNAs or shRNAs corresponding to genes that are amplified or deleted in human tumors, and we select for those constructs that promote tumorigenesis following transplantation into recipient mice. Initial efforts surveyed nearly 400 recurrently deleted genes in human hepatocellular carcinoma (HCC) and identified 12 new tumor suppressor genes. In a conceptually similar screen performed by Scott Powers (CSHL) using full-length cDNAs, we identified 10 new oncogenes in human HCC, including *FGF19* as a therapeutic target for existing neutralizing antibodies (Sawey et al. 2011). Ongoing work with Powers and colleagues is performing similar studies in ovarian cancer.

This year, we also completed an additional screen for novel tumor suppressor genes in lymphoma. We screened an shRNA library targeting genes deleted in human lymphomas to identify those whose suppression promotes tumorigenesis in a mouse lymphoma model. Among the new tumor suppressors we identified were adenosylmethionine decarboxylase 1 (AMD1) and eukaryotic translation initiation factor 5A (eIF5A), two genes associated with hypusine, a unique amino acid produced as a product of polyamine metabolism through a highly conserved pathway (see above). Through a secondary screen surveying the impact of all polyamine enzymes on tumorigenesis, we established the polyamine–hypusine axis as a new tumor suppressor network regulating apoptosis. Unexpectedly, heterozygous deletions encompassing AMD1 and eIF5A often occur together in human lymphomas, and co-suppression of both genes act to promote lymphomagenesis in mice. Thus, some tumor suppressor functions can be disabled through a two-step process targeting different genes acting in the same pathway.

Through our own genomics efforts, we also identified several new candidate cancer genes via next-generation sequencing. In a collaboration funded by the Monti Foundation with Dr. Steven Allen at North Shore/LIJ, we performed whole-exome sequencing on a unique mast cell leukemia. These studies identified potential driver mutations in the IgE mast cell receptor and the kit receptor that have implications for the treatment of patients with this disease. Other studies on AML have pointed toward new cancer genes that we are validating using mouse models.

Beyond identifying new activities of cancer relevance, our approach is revealing unexpected principles about the nature and organization of cancer genes. As examples, we were surprised that so many tumor suppressors are haploinsufficient, encode secreted proteins, or have pro-oncogenic activities in other contexts. We also did not expect recurrent amplifications and deletions to contain more than one relevant activity, yet our results imply this is the rule rather than the exception. Indeed, in a systematic study of genes recurrently located on 8p, we showed that shRNAs targeting multiple genes in this region could be tumor-promoting and that suppression of gene combinations showed that cancer-associated deletions contribute to cancer phenotypes in a manner that is distinct from single-gene mutations and should be considered and studied as distinct mutational events. We hope that further efforts will facilitate the functional annotation of the genomic alterations occurring in human cancers and identify vulnerabilities these lesions create.

Epilog

After 16 productive years at Cold Spring Harbor Laboratory, the Lowe laboratory moved to Memorial Sloan-Kettering Cancer Center in August 2011. During its tenure at CSHL, it expanded its research interests from focused studies on the action of p53 in apoptosis to more broad-based approaches for identifying cancer drivers and dependencies, thereby contributing to, and benefiting from, the great research environment at the Laboratory. Many students, postdocs, and technicians who were Lowe lab members throughout this period created a spirited and collaborative environment that impacted the social and scientific character of the Laboratory for nearly a decade. Moving forward, the Lowe laboratory will continue its association with CSHL through several formal and informal programs that have great potential to fuel exciting advances in cancer research during the coming years.

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REPRESENTATION METHYLATION SEQUENCING ANALYSIS

R. Lucito N. Cutter M. Vigliotti
N. Dimitrova K. Wrzeszczynski
E. Lum

In addition to genetic mutation, such as amplification and deletion, epigenetic aberrations are frequent events that can have far-reaching affects 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. Presently, our approach is to sequence small MspI fragments of the genome.

We have presently sequenced four tumors and two normal samples and have more tumors and normal samples in the queue. We sequenced two of the samples on two different formats of the Illumina sequencers and determined the coverage of each; we have switched to the newest platform. We developed sequencing pipelines for the analysis of the data and determined the number of reads necessary to account for 85% of the CpG sequences in the genomic island sequences. We are presently further analyzing the data with the goal of identifying new regions that can serve as markers for diagnosis and also as a discovery tool to identify gene candidates that are involved in therapy response. To do so, we have a tumor set that has been stage normalized to contain stages 3 and 4 tumors with accurate clinical data for the past 10 years. These 40 samples will be methyl sequenced and further analyzed.

Genomic Analysis of Ovarian Cancer

K. Wrzeszczynski, M. Vigliotti, E. Lum, N. Cutter, N. Dimitrova

In the United States, there will be ~22,000 new cases of ovarian cancer in 2011. Of those, ~14,000 will succumb to the disease. To better treat these women, and improve survival, our goal is to determine the molecular changes that have occurred in the patients' tumors and to interpret the significance that these changes have for the growth and development of the tumor. This aberrant growth is a result of chromosomal abnormalities and epigenetic variations. In addition, generally low rates of somatic nucleotide mutation in ovarian cancer as compared to other solid tumors suggest an increased significance of copy-number and epigenetic aberrations. This type of regulation has been shown to affect the many tumor suppressors and oncogenes discovered in ovarian cancer. The identification of genetic and epigenetic alterations from primary tumor cells has become a common method to identify genes critical to the development and progression of cancer. We have performed a bioinformatic analysis of copy-number variation and DNA methylation covering the genetic landscape of ovarian cancer tumor cells. We individually examined the copy-number variation and DNA methylation for 42 primary serous ovarian cancer samples using our MOMA-ROMA technology and 379 tumor samples analyzed by the Cancer Genome Atlas. We have identified 346 genes with significant deletions or amplifications among the tumor samples. Using associated gene expression data, we predict 156 genes with significantly altered copy number and correlated changes in expression. Among these genes, *CCNE1, POP4, UQCRB, PHF20L1,* and *C19orf2* were identified within both data sets. We were specifically interested in copy-number variation as our base genomic property in the prediction of tumor suppressors and oncogenes in the altered ovarian tumor. We therefore identify changes in DNA methylation and expression for all amplified and deleted genes. We predicted 615 potential oncogenes and tumor suppressor candidates

by integrating these multiple genomic and epigenetic data types. Genes with a strong correlation for methylation-dependent expression exhibited at varying copy-number aberrations include *CDCA8, ATAD2, CDKN2A, RAB25, AURKA, BOP1,* and *EIF2C3.* We provide copy-number variation and DNA methylation analysis for more than 11,500 individual genes covering the genetic landscape of ovarian cancer tumors. We show the extent of genomic and epigenetic alterations for known tumor suppressors and oncogenes and also use these defined features to identify potential ovarian cancer gene candidates.

Resistance Mechanisms in Ovarian Cancer

N. Cutter, M. Vigliotti, K. Wrzeszczynski

Epithelial ovarian cancer is the leading cause of death from gynecological malignancies. Currently, platinum-based chemotherapy (such as cisplatin or carboplatin), coupled with a taxane-based drug such as paclitaxel, is the primary treatment for ovarian cancer. Approximately 25% of patients either present with or rapidly develop resistance to chemotherapy, and all recurrent tumors are resistant. Epigenetic modifications have been associated with tumor formation and progression and may contribute to therapy response. We have screened a number of genes and family members for methylation in resistant patients and not in sensitive patients. We show for one such gene *CHD3*, a member of the Mi-2 NuRD complex, that loss of expression is linked to chemoresistance. *CHD3* is silenced through an epigenetic mechanism in both ovarian cancer cell lines and primary ovarian tumors. Ovarian cell lines transcriptionally silenced for *CHD3* have an increased resistance to the chemotherapy drugs carboplatin and cisplatin. Additionally, these cells are more invasive, have migratory ability, and display a transformed epithelial-to-mesenchymal (EMT) phenotype. Taken together, this provides the first evidence for a role for *CHD3* as an important mediator of chemoresistance in ovarian cancer, and we hypothesize that EMT is one of the underlying mechanisms. Furthermore, *CHD3* might represent a response predictor and potential therapeutic target for predicting chemoresistance in this disease.

Genomic Alterations of Phosphatases

E. Lum, G. Fan, 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 is the MTSS1 protein that 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 short hairpin RNAs (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.

PUBLICATION

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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 E. Son

The overall goal of our research is to understand the genetic basis of human disease. In particular, we have focused on cancer and autism. We have made a number of discoveries that have identified key genes that regulate these diseases, revealing insight into the mechanism responsible. These findings have had a major impact in the scientific community throughout the world, and in several instances, have affected the way clinicians analyze and treat these genetic diseases. The following are our major discoveries:

- Identifying *p63* as a gene affecting development, cancer, and aging.
- Discovering *CHD5* as a gene that prevents cancer.
- Revealing the genetic basis of autism.

Identifying *p63* as a Gene Affecting Development, Cancer, and Aging

A number of years ago, we discovered a gene called *p63* that no one knew even existed. Although *p63* looked very similar to *p53*—a well-studied gene that is defective in over half of all human cancers—it was not at all clear how *p63* really worked. We found that *p63* is needed for development, as mice lacking it have malformations of the limbs, are lacking skin, and have cleft palate. This work provided a clue to clinicians searching for the cause of a human syndrome in which children have birth defects of the hands and feet, abnormal skin, and severe cleft palate. Our work showing that *p63* is needed for development had a major impact, as it is now known that defects in the *p63* gene are the cause of *seven different* human syndromes characterized by birth defects affecting the limbs, skin, and palate. These birth defects can be so severe that in utero screening for *p63* mutations is sometimes performed in the clinic. We are currently analyzing mouse models of these human syndromes in an effort to define their genetic and molecular basis.

My group also discovered that *p63* affects aging. *p63* is needed for stem cells to replenish the tissues of our body; when *p63* is depleted, rapid aging ensues. These features of aging include curvature of the spine, hair loss, and severe skin lesions, indicating that *p63* maintains youthfulness. However, there is a fine balance, as *p63* must be controlled at just the right level. Indeed, during the last year, we discovered that too much of a certain p63 protein (ΔNp63α) leads to carcinoma—the most prevalent type of human cancer (Keyes et al. 2011). Pathologists throughout the world routinely use ΔNp63α as a marker for diagnosing human cancers of the breast, skin, and prostate.

In contrast to the tumor-causing ΔNp63α mentioned above, we discovered that other classes of p63 proteins (TAp63 proteins) function in an opposite way: They *prevent* cancer. We showed that TAp63 proteins are able to shut down the growth of a tumor that has already developed, causing the tumor to shrink dramatically. Importantly, TAp63 is able to shut down tumor growth even when its sister protein, p53, is completely absent. This work stunned the p53 cancer community, because it had always been assumed that having ineffective p53 was a virtual death sentence that would render any anticancer therapy ineffective. Our work shows this is not the case, as TAp63 can step in and do the job. We are currently working on strategies to activate TAp63 that might be feasible in the clinic.

Discovering *CHD5* as a New Cancer-Preventing Gene

Another major breakthrough in my laboratory was the discovery of *CHD5* as a tumor suppressor mapping to human 1p36—a region of our genomes that is sabotaged with deletions in cancer cells. Indeed, 1p36 deletions occur in many different types of human cancers, including those of the epithelia and brain, as well as those of the blood. Although the cancer community had searched for more than three decades for this tumor suppressor, identification of this gene presented a major challenge. My laboratory discovered *CHD5* as a 1p36 tumor suppressor. By generating mice with gain and

loss of the genomic region corresponding to 1p36 using chromosome engineering technology—a strategy that allows us to generate precise chromosome rearrangements in the mouse—we pinpointed a region of the genome with potent tumor suppressive activity. Using a series of genetic and molecular approaches, we identified *CHD5* as the tumor suppressor gene in the region and found that its product worked as a "circuit breaker" for a cancer-preventing network. In addition, we discovered that *CHD5* was frequently deleted in human glioma. Our work identifying *CHD5* as a tumor suppressor has had a major impact in the cancer field, as it is now known that *CHD5* is mutated in human cancers of the breast, ovary, and prostate, as well as in melanoma, glioma, and neuroblastoma. Furthermore, recent reports indicate that *CHD5* status is a strong predictor of whether anticancer therapy will be effective; indeed, patients with high levels of *CHD5* have much better overall survival than those with low levels.

Revealing the Genetic Basis of Autism

Our discovery during the past year was the finding that one of the most common genetic alterations in autism—deletion of a 27-gene cluster on chromosome 16—causes autism-like features (Horev et al. 2011). By generating mouse models of autism using chromosome engineering (the same technology that we had used for cancer gene discovery, discussed above), we provided the first functional evidence that inheriting fewer copies of these genes leads to features resembling those used to diagnose children with autism. First, "autism" mice had behaviors such as higher activity, resistance to change in environment, sleeping problems, and repetitive/restrictive behavior; each of these features is similar to clinical criteria used to diagnose autism in humans. Second, these mice had distinctive architectural changes in the brain that were detectable by magnetic resonance imaging (MRI). Our work provides functional evidence for the genetic basis of autism that was not previously appreciated. We believe that these mice will be invaluable for pinpointing the genes responsible for the autism-like features and for elucidating how these alterations affect the brain. They could also be used for designing ways to diagnose children with autism before they develop the full-blown syndrome, as well as for designing clinical interventions.

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Alea Mills (*center, left*) and lab members

CANCER GENES

S. Powers C. Eifert A. Mofunanya S. Sayers
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J. Li K. Revill

Our laboratory is one of the four research laboratories located further afield at the Woodbury Genome Center. This center was opened a little over 10 years ago to make the most of the inimitable opportunities arising from completion of the Human Genome Project (HGP) in 2001. By providing the first complete human genome sequence, the HGP enabled the development of vastly superior tools than had previously existed for detecting changes in the human genome. These superior tools were rapidly applied to cancer, which has long been known to be at its most fundamental level a genetic disease. What these tools have revealed in the last decade is that individual cancer genomes are disrupted in incredibly diverse and complex ways, essentially meaning that each tumor has its own unique set of altered genes. This explains how the behavior of individual tumors—how they progress and eventually respond to therapy—is so tremendously varied and difficult to predict.

Occasionally, cancer genome analyses have stumbled across previously unrecognized genetic alterations that are frequently found in the tumors of one particular tumor type, such as mutational activation of *BRAF* in most melanoma patients, that stand as a counter example to the overall diversity of mutations and that have provided a foundation for the development of new targeted therapeutics. However, for the most part, the cancer genome projects modeled after HGP, despite being tremendously successful at generating large lists of mutant genes and other alterations in human tumors, have provided very few new therapeutic strategies. However, the most surprising event of the last decade in cancer genome research has been the revolution in next-generation sequencing and the realization that within the next decade, next-generation sequencing will be routinely performed as part of cancer diagnosis, fueled by the hope that having the complete cancer genome and transcriptome will help doctors make the most informed treatment recommendations. Ironically, this will happen before the cancer genome projects have provided more than a small handful of new treatments. There is a tremendous gap that needs to be filled if cancer genome projects are to fulfill their promise of transforming cancer treatment.

Our view is that filling this gap will require a synthesis of cancer genome characterization with functional tests derived from the large number of model systems that have been developed in the past 100 years. There are so many model systems to test the relevance of a given genetic alteration to cancer, and there is really no excuse for not being able to translate genomic discoveries into new insights into what is biologically driving cancer progression. With new insights into biological drivers we can propose new therapeutic targets, which could in time be translated to cancer medicine. The cancer models are there, they just have to be chosen carefully and applied systematically.

Cancer Genome-Focused Screening

During the last seven years, we and our colleagues have developed an approach to discovering new treatment strategies that focuses directly on the genes most significantly affected by a given type of genetic alteration, e.g., gene amplification, in a given cancer type, e.g., liver cancer. This focused approach directly tests genes for tumor-promoting function in an appropriate cell type (Fig. 1). In a study we published this year, 124 amplified genes in human hepatocellular carcinoma (HCC) were screened in pools for oncogenicity by forced overexpression in a transplantable mouse hepatocyte model. 18 of these amplified genes turned out to be tumor-promoting "driver" genes, whereas the other 106 genes appeared to be inactive passenger genes. Interestingly, the correlation of mRNA expression with DNA amplification was unable to distinguish drivers from passengers in this functionally vetted set, indicating that passengers and drivers are both overexpressed as a result of DNA amplification; however, an algorithm that combined a functional interaction network described with a knowledge-based oncogenicity ranker (http://cbio.mskcc.org/tcga-generanker/) was able to distinguish drivers from passengers (albeit only partially). Importantly, a follow-up study using RNA interference (RNAi) established that HCC cells harboring an 11q13.3 amplicon were selectively dependent on the am-

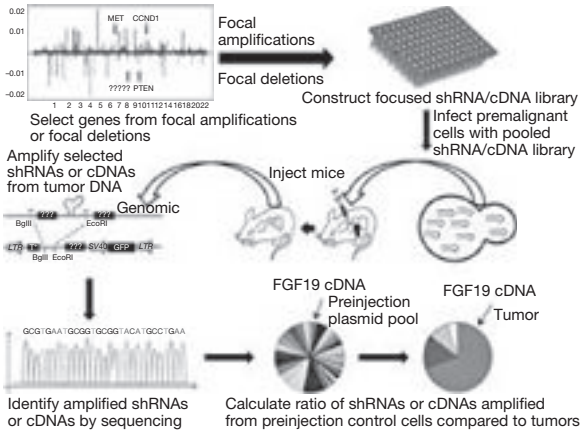

Figure 1. Cancer-genome-focused screening directly tests genes for tumor-promoting function in vivo. In this example, whole-genome copy-number alteration data obtained from tumor samples are computationally analyzed to nominate candidate driver oncogenes within focal amplifications and tumor suppressors within focal deletions. Next, focused shRNA or cDNA libraries are constructed from commercially available plasmid collections in order to overexpress the predicted oncogenes (cDNA library) or knock down the tumor suppressor genes (shRNA library). Premalignant cells are infected with pools of the focused library, which are then screened in the mouse for tumor promotion. Genomic DNA is then isolated from resulting tumors and shRNA or cDNA sequences are recovered using polymerase chain reaction (PCR) amplification. DNA sequencing is used to identify individual shRNA or cDNAs responsible for tumor promotion by comparison to the shRNA or cDNA composition of preinjected control cells.

plified driver gene and FGF19. This selective dependency was also observed using a monoclonal antibody directed against Fgf19, pinpointing a new targeted therapeutic strategy.

This approach is readily generalizable. It was modified to discover driver genes from recurrently deleted regions in human HCC, by screening pools of short hairpin RNAs (shRNAs) targeting the candidate deleted tumor suppressor genes, and modifications of the approach have been used to screen focused libraries in transplantable mouse models of lymphoma and leukemia.

The cancer genome-focused screening approach draws strength from the simple fact that it was designed solely to directly address the oncogenic relevance of human cancer genomic alterations. Another strength of the cancer genome-guided screening approach is that by focusing on fewer genes, the depth and strength of the corresponding RNAi library can be better optimized.

Our cancer genome-focused screening efforts are part of the National Cancer Institute (NCI)-sponsored Cancer Target Discovery and Development Consortium (http://ctd2.nci.nih.gov/) that was developed to incorporate cancer genome characterization into molecular target discovery and validation programs. The Cancer Target Discovery and Development (CTD2) network is the first coordinated effort aimed at translating that therapeutic potential for cancer patients. Five projects (http://ocg.cancer.gov/programs/ctdd.asp) focus on the development of novel scientific approaches to accelerate the translation of the genomic discoveries into new cancer treatments. The network emphasizes interaction of laboratories with complementary and unique expertise, including bioinformatics, genome-wide loss-of-function screening and targeted gain-of-function candidate gene validations, intelligent use of mouse-based screens, and small-molecule high-throughput screens. Within CTD2, we are currently performing cancer genome-focused screens in ovarian and other select cancers.

Pooled shRNA Screening for Cancer Targets

Since 2005, our lab has devoted much of its effort to genome-scale RNAi screening, which holds the promise of not only finding the best targets for cancer therapeutics, but also systematizing the search for combination therapeutic strategies. We have performed one screen to look for targets to inhibit in combination with inhibition of the mitotic regulator polo-like kinase (PLK1) in lung cancer, and another screen to find targets to inhibit in combination with a candidate cancer drug that combines oleic acid with lactalbumin ("Hamlet"); results from both of these efforts were published this year. We have also performed an RNAi screen to find targets to inhibit in combination with temozolomide, an alkylating agent used to treat glioblastomas and melanomas, and another screen to find targets to inhibit in combination with a phosphoinositol-3 kinase (PI3K) inhibitor. We have also screened HCC cells to look for new therapeutic targets under both normal conditions and the hypoxic conditions that more closely resemble how tumors grow in patients. Although in each case we have discovered some interesting "hits," the problem that we have been most frustrated with is the false-negative problem, resulting from ineffective shRNAs that do not silence their target gene. This prevents one from being able to use the extensive data sets that are gathered to "dial-in" on the function of individual genes, and instead limits shRNA screening utility to finding one or more genes that, once individually validated, can be pursued in detail. Once truly validated shRNA libraries have been developed, there is no doubt the technology will be much more broadly useful.

Genomic Analysis of Hepatocellular Carcinoma

With the ascendancy of centrally organized, productive, and well-funded cancer genome projects, which have already characterized thousands of cancer genomes, there corresponds a diminishing rationale for small individual laboratories to try to do the same. In what is likely to be our last cancer genome characterization project, we integrated analyses of the human hepatocellular DNA methylome with its copy-number alterations and transcriptional changes. We found two types of hepatocellular carcinomas (HCCs). In one case, we found that the DNA methylome is remarkably invariant and closely resembles normal fetal liver in the hypermethylation and silencing of liver-differentiation-specific genes. We showed that this state is associated with stem-like tumors that harbor few DNA copy-number alterations and that resemble fetal liver in the coordinated hypermethylation and silencing of tumor suppressors (e.g., *MST1* and *HNF4A*) as well as the hypomethylation and expression of oncogenes (e.g., *STAT5A* and *OPN*). In the other case, the HCC DNA methylome is markedly more variable and involves hypermethylation of polycomb target and developmental genes. This more variable epigenetic state was found in differentiated tumors expressing mature hepatocyte markers and containing extensive DNA copy-number alterations. Thus, destabilization of DNA copy number and the DNA methylome may both be necessary to induce tumorigenicity in mature hepatocytes. In contrast, stem-like HCCs can emerge from a fetal-like normal progenitor with both a stable genome and stable epigenome.

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Scott Powers (*standing, right*) with lab members

GENOMIC ANALYSIS OF CANCER AND HUMAN GENETIC DISORDERS

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The Wigler and Hicks group studies human cancer and genetic disorders from a population genomics perspective. The cancer effort focuses on breast and prostate cancer (the latter jointly with Lloyd Trotman) and involves collaborative clinical studies (with local, national, and international collaborators) to discover mutational patterns predicting treatment response and outcome. We also develop methodology for single-cell genomic and RNA analyses to detect cancer cells in bodily fluids such as blood and urine. This last has major potential applications to the early detection of cancer and monitoring its recurrence and response to therapy during and after treatment. The single-cell analysis has also led to insights about the clonal evolution and heterogeneity of cancers (Navin et al. 2011). This work may lead to a better understanding of initiation, progression, and metastasis and shed light on the stem cell hypothesis of cancer and host responses.

Our genetic efforts are largely focused on determining the role of new (de novo) mutation in pediatric disorders with a strong genetic contribution. We study autism as part of a large study of simplex families organized by the Simons Foundation (Fischbach and Lord 2010), as well as congenital heart disease with Dorothy Warburton of Columbia University and pediatric cancers with Ken Offit of Memorial Sloan-Kettering Cancer Center (MSKCC). In collaboration with Alea Mills, we helped create a mouse model for one of the most common genetic abnormalities contributing to autism (Horev et al. 2011). Recent work has confirmed and extended our previous observations on the role of de novo copy-number variation (large-scale mutation) in autism (Levy et al. 2011), with similar results in the other disorders. In a large-scale exome sequencing project in collaboration with Dick McCombie (at the Genome Center at CSHL) and the Genome Sequencing Center at Washington University in St. Louis, we have proven the role of small-scale de novo mutations that disrupt genes in autism (Iossifov et al. 2012). Overall, our results succeed in confirming our previous genetic models for autism incidence and identify strong candidate mutational targets, from which biological models of autism can be made and tested.

Breaking with tradition, the remainder of this report contains two position statements on the direction in which we are taking our science, and why.

Applications of Single-Cell Analysis to Biological and Medical Problems

For several years, we have explored methods for gathering and analyzing nucleic acid sequences from single cells. There are at least five broad areas in which single-cell methods can be applied: (1) cancer, (2) neurobiology, (3) disorders of stem cell renewal, (4) detailed dissection of cell-state transitions, and (5) genome assembly. We are working on each. All of these applications require improvements in the gathering of single-cell data, both its quality and cost, and developing new processing algorithms. Some of the mathematical challenges are new. To improve data generation, we make use of DNA barcoding to facilitate massively parallel sample processing, increase the uniformity of data quality, and reduce costs.

The applications of single-cell analysis in cancer are nearly endless. Single-cell genome analysis can be used for detecting cancer cells in samples such as blood or urine obtained by noninvasive or minimally invasive procedures. A more thorough analysis of standard biopsy material, such as needle biopsies, can be achieved by single-cell genome profiling, revealing details of cancer heterogeneity, subpopulation structure, host reaction, and the presence of cancer cells in surgical margins and lymph nodes. Through single-cell RNA expression, the host response at the sites of cancer growth can be monitored: the presence of capillary endothelium, immunocytes of all types, and reactive stroma. The identity of soluble factors to which these cells are responding may be inferred by observing their response signatures. By means of statistical methods correlating clinical outcomes with single-cell observation, we shall be able to improve detection, prognosis, therapeutic choice, and monitoring response to therapy. The most transformative application would be a blood test for early detection of cancer onset, where the

DNA analysis provides evidence for the presence of cancer cells and disease stage, and the RNA analysis indicates the cancer's tissue origin and subtype. Both together can aid in prognosis and choice of therapeutic intervention. Finally, many fundamental questions of cancer biology can be addressed by single-cell analysis. Among these: How do cancer populations evolve? Is there in fact a stem cell population that is genetically distinct from the majority of the tumor population? From what cell or combination of cells do metastases arise? To what extent and through what processes do cancer cells cooperate?

Single-cell expression analysis can also be used to develop a deeper understanding of neural and nonneural subtypes in the nervous system (brain, chord, and gut) where tissue heterogeneity obscures bulk analysis. The result can be a better modeling of neuronal networks through identification of neuronal subtypes and a better understanding of the response of neuronal cells to stimuli. The role of somatic mutation in nervous system disease can be explored. Finally, these methods can help refine our knowledge of the fates of pluripotent stem cells induced to differentiate into neuronal cells. If successful, we could explore the functional consequences of candidate gene mutations.

The identity of stem cells and the role of aberrant stem cells in medical disorders are still highly unexplored. By an iterative process coupling cell separation and regenerative assays, single-cell RNA analysis can help identify which cells in a tissue or in distant tissues are in fact the cells with regenerative capacity. Several medical disorders, particularly those of aging, may be the result of somatic mutation in stem cells causing the replacement of critical cell types over time with dysfunctional versions. The types of disorders that might be caused by this mechanism include a wide variety of neurodegenerative conditions (e.g., Alzheimer's), disorders of autoimmunity (e.g., lupus), cardiomyopathies, and a variety of cutaneous (e.g., psoriasis) and connective tissue (e.g., scleroderma) diseases. The mechanisms underlying these disease processes might become apparent by combining DNA and RNA analysis of single cells, looking for aberrant genomes or expression patterns in stem cells. The latter may be identified at the single-cell level in complex organs, thereby overcoming the problems that beset the analysis of mixed-cell populations.

Examples of cell-state transitions abound in all living systems: the progression through the cell cycle, response to nutrient opportunity, differentiation, responses to growth factors and other signaling molecules, and even responses to contact with other cells. Often, these transitions involve a causal sequence of changes in the expressive state of the cell, the proteins and RNAs that they make. Because these changes typically occur in cell populations that are not in temporal synchrony, the study of the sequence of changes often cannot be determined with precision. Single-cell analysis offers one way around this problem, as each cell represents a snapshot in time at the point when it is destroyed and converted to an ensemble of macromolecules. In principle, the overall series of changes can then be assembled into a coherent whole provided that (1) there is some overlapping signature in the macromolecular composition between time-adjacent states and (2) the temporal series is relatively similar from cell to cell.

We can make sequence libraries from a fraction of a cell genome. Genome analysis itself can be improved by doing this. If the DNA of a cell is diluted sufficiently into isolated "packets," any contiguous region we sequence from a given packet is likely to represent only one of the two parental haplotypes. If the DNA is not broken into small pieces during dilution, but diluted in large blocks—even if we subsequently break the DNA into small pieces when we make libraries—we can reassemble the short-read sequences into large haplotype blocks. The result, if conducted efficiently using many cells as a starting point, would ultimately be a haplotypic assembly of the genome of any organism at roughly the cost of sequencing the entire organism in the conventional manner. As a practical matter, this converts the most efficient sequencing apparatus currently available, which is a short-read apparatus, into a long-read single-molecule apparatus.

Genetic Models of Autism

A large proportion of our lab works on autism genetics. The purpose of these notes is to give some scientific perspective to what the group has achieved and the future direction it will take. The rewards of studying autism from a genetic point of view are great. The disorder affects some of the most profoundly human traits. A complete analysis may reveal new sources of phenotypic variation. A good set of targets provides guides to therapies. And there are unmet needs for early detection and counseling families and individuals coping with the condition.

It was evident to some by as early as the mid 1980s that autism was likely to be a disorder caused by new mutation. The disorder was variable, hinting at multiple underlying causes; the incidence was high; the her-

itable component was appreciable but low; but the concordance in identical twins was higher than in any other cognitive or behavioral impairment. Human geneticists had failed repeatedly to produce evidence of heritable underlying events, except in the limited cases of X-linked disease. In both of these cases, Fragile-X syndrome (FXS) and Rett syndrome, new or recent de novo mutations were clearly the root cause. Geneticists using transmission genetics would explain their failure under the rubric of a "complex" disorder, caused by subtle interactions of multiple genes that would therefore be hard to crack. But what we knew in fact pointed to the existence of singular events of strong penetrance. Cytologic analysis had showed that many rare large-scale chromosomal events could result in developmental anomalies. Cytology could only scratch the surface of the richness of the consequences of new mutation.

In the early 2000s, methods for examining copy-number variation (CNV) developed in our lab (Lucito et al. 2003) became very powerful when combined with the data of the human genome sequence assembly (Healy et al. 2003). This led to the discovery of widespread CNV in the human gene pool (Sebat et al. 2004) and the hope that we could demonstrate autism was associated with new mutations likely to disrupt the dosage of functioning genes. Supported by the Simons Foundation, we succeeded in showing this (Sebat et al. 2007), and our work fed the acceptance of the idea that disorders associated with drastically reduced fecundity would be often caused by new mutation through the action of gene dosage effects. Altering the functional dosage of certain genes, whether by duplication, deletion, or disruption, would manifest upon transmission as dominant traits, and we sought evidence for this in the AGRE data of multiplex families. We found the evidence (Zhao et al. 2007), namely, that boys born to parents with two previous offspring with autism spectrum disorder (ASD) had a 50% chance of being on the spectrum. These observations were confirmed in independent studies by ourselves and others.

Mathematical modeling of the family risk function using the AGRE data, and data from other sources, led to several predictions (Zhao et al. 2007). Up to half of autism might be explained by new mutation, and a large part of the remainder would be due to transmission of strongly penetrant variants carried by asymptomatic parents. We speculated that most often the carrier parent would be the mother.

Subsequent work has focused on defining the list of autism target genes and developing a more quantitative genetic model. We seek a list of target genes in the expectation that such a list would provide medical geneticists with improved tools for diagnosis, especially early diagnosis, and also yield insights into physiologic mechanisms and thus ideas for intervention. Our most recent studies of autism were based on the Simons Simplex Collection, an unparalleled sample set, and are published in *Neuron* (Gilman et al. 2011; Iossifov et al. 2012; Levy et al. 2011). These papers provide a leap forward in assembling the list of gene targets (Gilman et al. 2011), validated the role of de novo CNV in autism (Levy et al. 2011), and provided evidence of a roughly equal role of transmission of rare CNV. Levy et al. (2011) provided evidence that the autism candidate loci contained a functionally convergent network of genes, and they extracted a list of the most likely autism candidate loci from the CNV data. Iossifov compared sequence data within families to find compelling evidence that de novo mutations which disrupt genes contribute to autism. Many of the findings of Iossifov are found in three smaller studies published essentially concurrently (Neale et al. 2012; O'Roak et al. 2012; Sanders et al. 2012). Recurrence analysis confirmed previous estimates that there are on the order of 200–800 (most likely, 350–400) dosage-sensitive genes that when disrupted can contribute significantly to autism. Most significantly, the list of genes showed a strong overlap with genes that encode proteins whose translation may be under the control of FMRP, the product of the gene responsible for fragile-X syndrome (FXS). Nearly half of autism dosage-sensitive genes may be so controlled. FMRP is one of the central regulators of synaptic plasticity, the physiologic mechanism underlying the response of neural networks to repetitive stimuli.

In addition to producing an extensive list of well-vetted autism gene targets, we have focused on obtaining a better, more detailed quantitative genetic model for autism incidence. What answers would such a model provide, and why would they be useful? A detailed quantitative model would determine with greater precision the number of dosage-sensitive gene targets and determine more precisely the overall role of these in incidence. Without such information, it will be hard to know how much of causation we are missing, and whether we need to consider extragenic mechanisms to explain phenotypic variation. Without such a model, we would be unaware of whether we were dealing competently with the problem. An adequate model would explain the role of gender bias in transmission, and whether certain targets are gender-specific. Properly understood, gender bias might guide thinking about ther-

apy. At the present time, there are gaps in the evidence supporting any model or mixture of models. Filling those gaps either with further evidence or by correcting the models will be valuable contributions to our understandings of genetics and cognition.

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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 contributions of the microenvironment in which the cancer cells arise and live. Solid tumors are abnormally organized tissues that contain not only cancer cells but also various stromal cell types and extracellular matrices, and these latter components constitute the microenvironment. Communication between the different components of the tumor influences its growth, response to therapy, and ability to metastasize. The lab studies the importance of such communications 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 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 cytotoxic immune responses against tumors. Egeblad is interested in how different types of myeloid cells are recruited to tumors and how their behaviors (for example, their physical interactions with cancer cells and other immune cells) influence cancer progression including metastasis. The lab is also 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 may 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 novel paradigm for considering this aspect of cancer biology. Using sophisticated model systems such 3D cell culture platforms and transgenic mice, his team found that a protein called Scribble normally regulates proper differentiation of breast epithelial cells, coerces them into the correct organization and shape, and enforces resistance to cancer. They also have found that deregulation of the Scribble pathway results in the development of undifferentiated tumors in mice. Muthuswamy's team determined 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 identify therapies aimed at preventing precancerous lesions from becoming invasive. This past year, the lab has collaborated with the Tonks lab to develop novel approaches for treating HER2-positive breast cancers.

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 through hundreds of thousands of experi-

mental 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.

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 the reasons why cells in certain cancers are responsive to the inhibition of one particular gene or gene product. For instance, why do non-small-cell lung cancer (NSCLC) cells that have a particular mutation in the EGF (epidermal growth factor) receptor respond dramatically to its inhibition by the drug Tarceva (erlotinib). This occurs in 15%–20% of patients, in whom the great majority, within 1–3 years, develop resistance. Various mutations have been implicated in about half of resistant patients. Sordella and colleagues have discovered a new resistance mechanism in a subpopulation of NSCLC cells that are intrinsically resistant to Tarceva. These tumor cells were observed to secrete elevated amounts of a growth factor called transforming growth factor-β (TGF-β), which in turn increases secretion of interleukin-6 (IL-6), an immune signaling molecule. Significantly, these effects were independent of the EGF receptor pathway. The team therefore hypothesized that inflammation is one factor that can render a tumor cell resistant to treatment with Tarceva. In other work, Sordella collaborates with the Krainer lab to study whether alternative splicing has a role in the failure of p53-mediated senescence to halt oncogenesis in certain lung cancers.

Nicholas Tonks and colleagues study a family of enzymes called protein tyrosine phosphatases, or PTPs, which remove phosphate groups from proteins and other signaling molecules, such as lipids, in cells. Disruption of PTP function is a cause of major human diseases, and several of the PTPs are potential therapeutic targets for such diseases. Tonks' group seeks to characterize fully the PTP family, understand how their activity is controlled, how they modify signaling pathways, and how those pathways are abrogated in serious illnesses, including cancer, diabetes, and Parkinson's disease. The overall goal is to identify new targets and strategies for therapeutic intervention in human disease. This year, Tonks and colleagues have defined new roles for PTPs in regulating signaling events in breast cancer, identifying three PTPs as novel potential tumor suppressors. They have characterized the regulation of PTP1B by reversible oxidation, demonstrating that it is regulated by covalent modification of the active site by hydrogen sulfide (H_2S) under conditions of endoplasmic reticulum (ER) stress that are linked to protein-folding-related pathologies, such as Parkinson's and Alzheimer's. In addition, they have generated recombinant antibodies that recognize selectively the oxidized conformation of PTP1B; these antibodies display the ability to promote insulin signaling in cells and suggest novel approaches to therapy for diabetes. Finally, they have also discovered a novel mechanism for allosteric regulation of PTP1B activity, offering the possibility of developing small-molecule drugs that could inhibit the phosphatase and thereby modulate signaling by insulin and the oncoprotein tyrosine kinase HER2—potentially offering new ways to treat insulin resistance in type 2 diabetes and breast cancer.

Lloyd Trotman's recent research path traces to his discovery some years ago 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 established 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. Now, the Trotman lab is generating mouse models that accurately reflect the core genetic changes driving human metastatic prostate cancer, with the aim of developing novel molecular assays that separate the few men who are at risk of developing lethal disease from the rest. Last year, Trotman and colleagues iden-

tified a new prostate tumor suppressor gene called *PHLPP1* and showed that it acts in concert with *PTEN*, a well-studied tumor suppressor that is mutated in roughly half of prostate cancer patients. The Trotman lab is using these findings to establish prediction methods for disease progression, based on high-resolution genomic analysis techniques developed at CSHL by Drs. Wigler and Hicks. The lab is also studying regulation of *PTEN* stability and nuclear transport, because 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 to control cellular growth, differentiation, and morphogenesis. Alterations affecting Ras and Rho functions are involved in cancer and various neurodevelopmental disorders. Van Aelst's team has extended its prior 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. In addition to a role for *OPHN1* in activity-driven glutamatergic synapse development, they have obtained evidence that *OPHN1* has a critical role in mediating mGluR-LTD, a form of long-term synaptic plasticity, in CA1 hippocampal neurons. Their findings provide novel insight not only into the mechanism and function of mGluR-dependent LTD, but also in the cellular basis by which mutations in *OPHN1* could contribute to cognitive deficits in patients. In addition, the Van Aelst team has discovered a critical role for a novel activator of Rho proteins in the genesis of cortical neurons. Defects in cortical neurogenesis have been associated with cerebral malformations and disorders of cortical organization. The team finds that interfering with the function of the Rho activator in neuronal progenitors in embryonic cerebral cortices results in an increase in the number of proliferating neuronal progenitors and defects in the genesis of neurons. This provides novel insight into mechanisms that coordinate the maintenance of the neural progenitor pool and neurogenesis.

The research of Hongwu Zheng and colleagues aims to define the complex biology of malignant glioma pathogenesis, with the ultimate goal of translating the developed knowledge into patient benefits. Although eerily similar in terms of their self-renewal capacity and distinct phenotypic plasticity, malignant glioma cells conspicuously lack the terminal differentiation traits possessed by their normal counterparts—neural progenitors. Using multiple approaches combining human cancer genomics, animal modeling, and stem cell biology, Zheng has unraveled the causal relationship between aberrant differentiation and ensuing gliomagenesis. Perhaps more importantly, his team has demonstrated that forced restoration of differentiation capacity within glioma cells can drastically attenuate their tumorigenic potential. This finding fits well with the team's overall strategy, which is to target differentiation control pathways as a novel avenue for malignant glioma treatment. To this end, they have sought to (1) develop various animal models to recapitulate the human glioma pathogenesis and use them to trace and investigate in vivo tumor initiation/progression and (2) identify key pathways/players controlling normal and neoplastic neural progenitor cell renewal and cell-fate determination.

THE TUMOR MICROENVIRONMENT

M. Egeblad H. Askautrud J. Cappellani M. Fein T. Kees E. Nakasone J. Park J.H. Park J. Qiu

Solid tumors are composed of cancer cells and stroma, a supportive framework that constitutes the tumor microenvironment. It includes the extracellular matrix (ECM) and stromal cells such as fibroblasts, adipocytes, cells of the vascular system, and immune cells. Interactions between epithelium and stroma are essential for normal organ development. As tumors develop and progress, they undergo dramatic changes that involve both the cancer cells and the microenvironment. Stromal components that have been proposed to have a more pronounced tumor-promoting function in advanced stages than in earlier stages include cells of the myeloid lineage, such as macrophages and dendritic cells.

We have developed an imaging method that permits real-time spinning-disk confocal microscopy of tumor–stroma interactions in mouse models of human breast cancer (Fig. 1). This allows us to follow, for example, vascular leakage, myeloid cell infiltration, and cancer cell proliferation and death in real time. We use this technology to understand how cancer is affected by the microenvironment.

Effects of the Tumor Microenvironment on Response to Cancer Therapy

When tumors do not respond to treatment, patients die. Understanding the mechanisms of therapy response and resistance is therefore of vital concern. Surprisingly, little is known about how cancer cells in intact tumors respond to classical chemotherapy, although these drugs have been used for decades. Most knowledge on the responses has been obtained from cell culture or xenograft animal experiments, but such experiments are often not predictive of drug responses in patients.

Development of resistance can be caused by cancer cell intrinsic factors (genetic or epigenetic changes) or by extrinsic factors such as survival factors secreted from stromal cells or impairment of drug penetration through the altered tumor ECM. Certain organs, such as the bone marrow and the thymus, have been shown to offer protection from chemotherapy mediated by secreted factors from stromal cells (e.g., interleukin 6). However, it is not known how the evolving microenvironment of solid tumors shapes drug sensitivity.

We are using in vivo spinning-disk confocal imaging to study drug sensitivity in the context of evolving tumor microenvironments. We have treated mouse models of mammary carcinoma with doxorubicin and followed drug responses by live imaging. Cell death started ~24 h after doxorubicin treatment, and cells died by necrosis and not apoptosis. We further showed that the microenvironment of different tumor stages participated in regulating the drug response. Early-stage lesions responded poorly compared to intermediate-sized, early carcinoma stage tumors. However, in vitro, sensitivity to doxorubicin was similar for cancer cells from different stages. Treatment also led to a new microenvironment, as dead cells recruited new myeloid-derived cells, changing the microenvironment of the responding tumors. Thus, imaging intact tumors acutely after treatment with chemotherapy revealed a complex and evolving relationship between microenvironment and the drug sensitivity of cancer cells.

Effects of Myeloid Cells on Breast Cancer Metastasis

The prognosis of metastatic breast cancer is poor, and although genetic changes that facilitate metastasis have been identified, such information has not yet improved patient outcomes much. More than a century ago, Dr. Stephen Paget observed that metastases develop preferentially in certain organs, suggesting that factors external to cancer cells influence metastasis. Indeed, it is now recognized that growth factors and cytokines secreted by myeloid-derived cells have a role in the formation of metastases.

Traditionally, studies on metastases have relied primarily on measurements made at the endpoint of the process, the establishment of micro- or macrometastases. However, the metastatic process is dynamic and characterized by the ability of cancer cells to move from

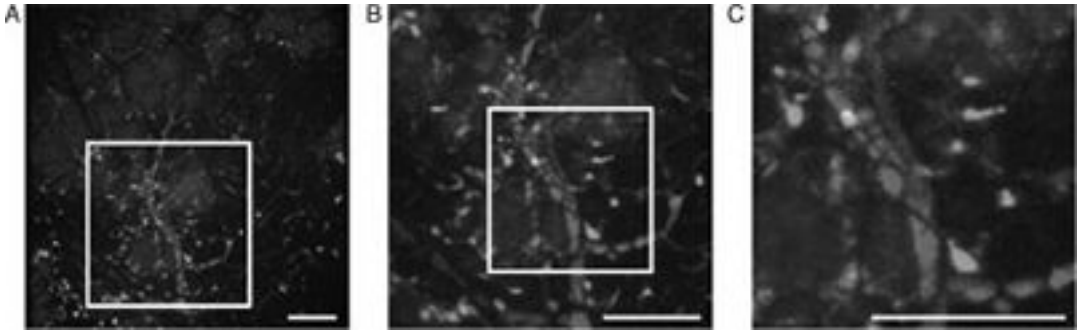

Figure 1. Imaging tumor microenvironments in live mice. The image size and the pixel resolution at the image is demonstrated using spinning-disk confocal microscopy on a live MMTV-PyMT;ACTB-ECFP;Fsp1$^{+/+.EGFP}$ mouse after intravenous injection with 70-kDa rhodamine-conjugated dextran. Cancer cells, fibroblasts, and vasculature are labeled. The same maximum intensity projection is shown at increasing magnification (*A*, *B*, *C*). Bars, 100 μm. (Adapted from Ewald et al. 2011.)

one part of the body to another: Cells exit the primary tumor, invade the local tissue, enter blood or lymphatic vessels, and are transported to a distant site where they exit the vessels and move into the tissue. Thus, a better understanding of metastasis requires technologies that can follow these dynamic processes in vivo.

Our laboratory is using spinning-disk confocal microscopy to determine how interactions between myeloid-derived cells and cancer cells influence metastasis. We are comparing the microenvironment of tumors formed from the metastatic 4T1 and the nonmetastatic 4T07 cell lines, isolated from the same breast tumor. We have identified significant differences in the types of chemokines that are secreted by the cancer cells and in the nature of the myeloid cell infiltrate between the metastatic and nonmetastatic tumors. Strikingly, tumors grow more slowly and metastasis is greatly reduced in mice that lack the receptor for one of the chemokines that specifically is secreted by metastatic cancer cells. Ongoing studies are addressing how the chemokine-chemokine receptor signaling axis between cancer cells and myeloid-derived cells influences metastasis.

Using Tumoricidal Activities of Macrophages

Tumor-associated macrophages have tumor-supporting activities, and their infiltration is associated with poor patient prognosis. However, macrophages are capable of killing tumor cells (Fig. 2) if they are activated with interferon-γ (IFN-γ) and agonists of receptors for pathogen-associated molecular patterns. We are using live cell imaging of cocultures between macrophages and cancer cells to understand the mechanisms responsible for the tumoricidal activities. Macrophage-mediated cancer cell killing is highly effective, specific for cancer cells, and requires direct cell–cell contact between macrophages and cancer cells. A potential mediator of the cell killing has been identified and is under further investigation.

Antigen-Presenting Myeloid Cells Stably Engage Tumor-Specific T Cells in the Tumor Microenvironment

This work was done in collaboration with Matthew Krummel, University of California, San Francisco. Antigen presentation in the tumor microenvironment should lead to activation of tumor-specific T cells and thereby cytotoxic activities against the tumor. Yet, although T cells with specific reactivity have been isolated from many solid tumors, the immune system is not capable of eradicating most tumors. To determine the potential inhibitory activities on T-cell responses, a fluorescent- and antigen-linked transgenic model of breast cancer was developed. This model enabled live imaging of antigen presentation in situ. The primary cells responsible for ingesting tumor antigens and presenting them to T cells were identified as low-motility myeloid cells, with properties of both dendritic cells and macrophages. Marker analysis further demonstrated that these antigen-presenting cells were a subset of the myeloid cells that previously have been implicated in tumor remodeling. These antigen-presenting cells localized along tumor margins and engaged in long-lived interactions with tumor-specific T cells. In vitro, these myeloid cells could capture cytotoxic T cells in signaling-competent conjugates, yet, the interaction

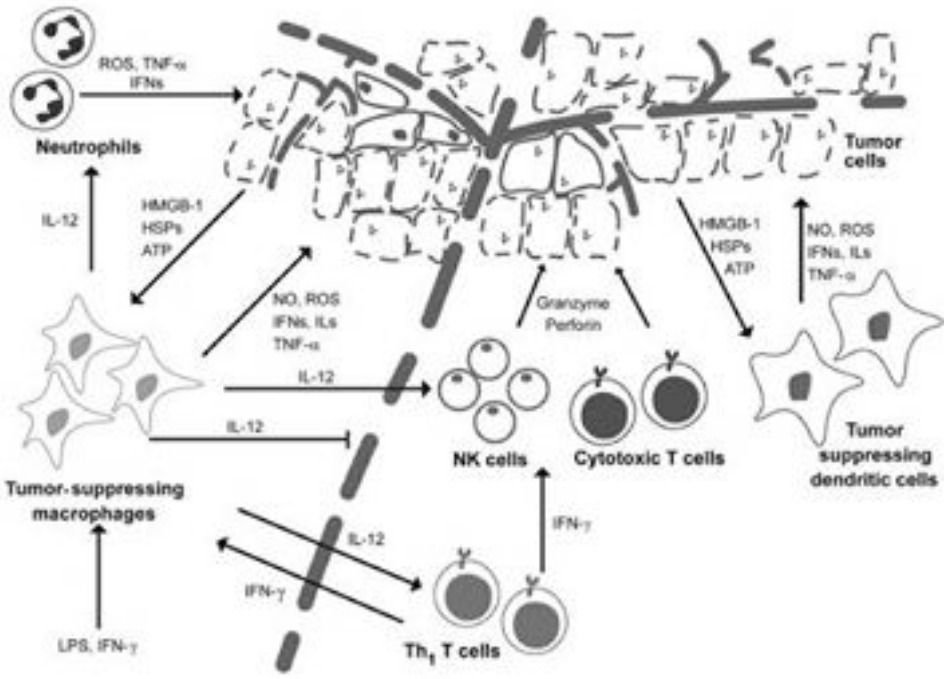

Figure 2. Tumor-suppressing functions of myeloid-derived cells. Macrophages can be stimulated with lipopolysaccharide (LPS) and IFN-γ to gain tumor-suppressing functions. These functions comprise the direct killing of tumor cells as well as the release of proinflammatory cytokines that inhibit angiogenesis and lead to the activation of natural killer (NK) cells, Th_1 cells, cytotoxic T cells, and neutrophil granulocytes. Factors released by dying tumor cells, such as ATP, can further enhance macrophage activation. (From Kees and Egeblad 2011.)

did not support full T-cell activation or sustain cytolysis of cancer cells. Thus, these antigen-presenting cells fail to stimulate T cells and may serve as a barrier to an effective T-cell response. This work demonstrated the behavior of tolerized T cells within tumors and identified a target for immunotherapies.

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

Y. Lazebnik J. Milazzo

During the last year, we continued to test a model, which we proposed previously, that some viruses can contribute to carcinogenesis and tumor progression by fusing cells. Two consequences of such fusion are of particular interest. One is an abnormal combination of properties in a cell that is derived from fusion of cells of different types, such as a transformed cell and a bone marrow stem cell. The hypothesis that such an outcome could be consequential to carcinogenesis was supported by recent findings that bone marrow stem cells fuse to differentiated cells in the body and reprogram the resulting hybrids into progenitors. Remarkably, it was also found that the rate of stem cell fusion increases many-fold in animals that were irradiated or suffered from chronic inflammation. Another consequence of cell fusion is chromosomal instability (CIN), which is a well-known feature of cell hybrids, although the mechanistic link between cell fusion and CIN is poorly understood. The facts that CIN is also a common feature of solid cancers and that cells of many of these tumors have increased ploidy suggested that cell fusion could be one of the processes that triggers CIN in premalignant cells.

During the last year, we worked in three directions: (1) We explored how oncogenes can regulate expression of retroviruses, (2) by collaborating with Alex Koulakov, a theoretical physicist, we set out to understand how properties of cell hybrids are determined, and (3) by collaborating with David Berman and Ming Tseh-Lin at Johns Hopkins University, we tested whether cell fusion occurs in human cancers.

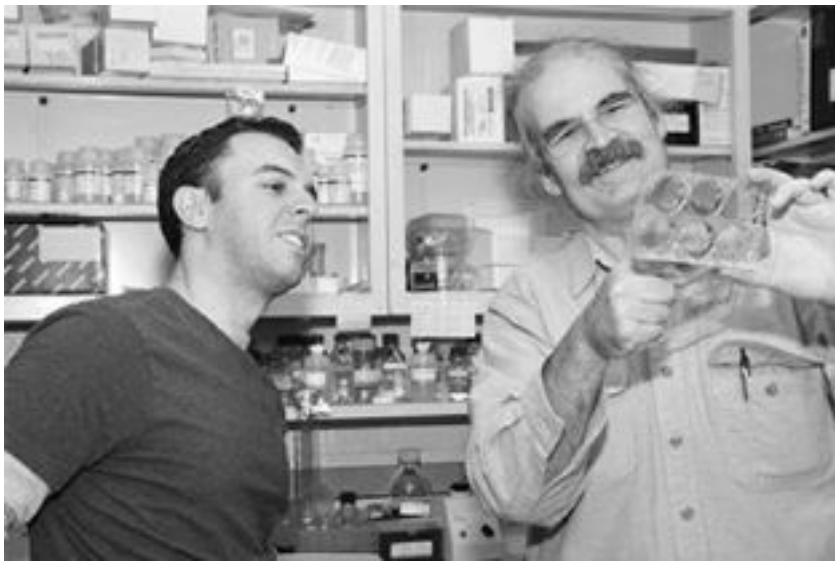
Our model implies that deregulation of fusogenic proteins can elicit cell fusion. We noticed that expression of genes from a retroviral vector is regulated by oncogenes *E1A* and *HRAS*, which is consistent with previous reports that expression of retroviral proteins is regulated by cellular transcription factors. We decided to explore this observation because all known human fusogenic proteins are of retroviral origin. However, this direction was terminated before it came to fruition due to lack of funds.

Our collaboration with Alex focused on the mechanisms that determine the ability of cell fusion to create new cell types, both normal and abnormal. How these properties are determined is largely unknown. Alex proposed that this ability can be better understood by considering cells as attractor networks, whose basic property is to adopt a set of distinct, stable, self-maintaining states called attractors. According to this model, fusion of two cell types is a collision of two networks that adopted distinct attractors. To learn how these networks reach a consensus that would define cell type of the hybrid, he modeled cell fusion computationally. To do so, Alex simulated patterns of gene activities using a formalism developed to simulate patterns of memory in neural networks. We have found that the hybrid networks can assume attractors that are unrelated to parental attractors. This observation implies that cell fusion can create new cell types by nearly instantaneously moving cells between attractors. We also showed that hybrid networks are prone to assume spurious attractors, which are emergent and sporadic network states. This finding means that cell fusion can produce abnormal cell types, including cancerous cells, by placing cells into normally inaccessible spurious states. Our model can explain previously puzzling properties of cell hybrids and predicts that using cell fusion as a therapeutic tool must be done with an abundance of caution.

A major outstanding question is whether cell fusion contributes to cancer in humans. The main difficulty with answering this is the lack of an approach that can distinguish a hybrid of two genetically identical cells from a cell produced by genome duplication. An alternative is to test whether cancers that arise in transplant patients contain hybrids between the cells of the recipient and the donor. Two groups of such patients are of interest: (1) recipients of bone marrow or circulating hematopoietic stem cells who subsequently developed cancers of solid organs and (2) recipients of solid organs who developed cancers in those organs. If cancers arise from fusion of circulating cells to cells of solid organs, cells of these cancers would carry genetic polymorphisms specific to the donor and the recipient, which can be determined with available tools. Our collaborator, Dr. David Berman at Johns Hopkins University, has located a small set of samples from cancers arising in the transplanted organs, and Dr. Ming

Tseh-Lin kindly agreed to analyze them for possible chimerism. We are awaiting the results of this analysis.

We have been unable to raise funds to support our research to the extent required to definitively test our model, but we hope that our results and ideas have helped to raise sufficient interest in testing the possibility that cell fusion has a role in cancer development and progression.



Joseph Milazzo and Yuri Lazebnik

EPITHELIAL CELL BIOLOGY AND CANCER

S.K. Muthuswamy D. Akshinthala B. Xue
M. Feigin

Cell polarization is a process by which cells establish and maintain asymmetric distribution of proteins and organelles. Cell polarity proteins have important roles in the establishment of cell polarity and regulation of tissue morphogenesis. Loss of tissue structure is a hallmark of cancer; however, the mechanisms by which tissue structure is disrupted and the role it has during cancer initiation and progression are poorly understood. We investigate the role played by cell polarity proteins during initiation and progression of carcinoma.

We have previously shown that the cell polarity complex Par6/aPKC is required for transformation of three-dimensional epithelial structures. The role played by Par-3, another member of the Par6/aPKC protein complex, has never been investigated. Recent genomic studies suggest that Par-3 is deleted in some cancers; however, the role Par-3 has in cancer is not known. We have discovered that dysregulation of Par-3 promotes metastasis by activating a Rac-mediated signaling pathway that disrupts cortical actin organization and inhibits E-cadherin junction maturation, which leads to a decrease in cell–cell cohesion. In particular, we find that expression of Par-3 in cell membranes is down-regulated both in human breast cancers and in lung metastasis of ErbB2 transgenic mice. Down-regulation of Par-3 cooperated with ErbB2 to induce invasion and metastasis in vivo. Interestingly, the metastatic behavior was not associated with an overt mesenchymal phenotype. However, loss of Par3 activated a Tiam1/Rac/GTP/IRSp53/WAVE2/Arp2/3 pathway that disrupted cortical actin, blocked E-cadherin junction maturation, and decreased epithelial cell–cell cohesion. Cortical actin and Arp2/3 localization were disrupted in tumor cells in vivo. Thus, we identify Par-3 as a novel regulator of metastatic behavior in tumor epithelial cells.

We have also discovered that down-regulation of cell polarity proteins induces phenotypic plasticity to mammary epithelial cells that allows them to either undergo normal morphogenesis or display invasive behavior in response to intrinsic or extrinsic signals. We found that dysregulation of polarity proteins can either cooperate with oncogenes (ErbB2 or KRasv12) or with changes in microenvironment (IL-6, TNF-α) to acquire the ability to invade in a bed of Matrigel/Collagen-1 extracellular matrix. Simultaneous loss of two polarity proteins is sufficient to induce invasive behavior, in the absence of expression of oncogene. Our results identify an unexpected and novel role for cell polarity proteins as regulators of phenotypic plasticity in epithelial cells. In doing so, we provide an insight into the mechanism by which tumor cells can acquire invasive potential early during the genesis of carcinoma. We believe that the ability of cells to acquire phenotypic plasticity complements the identified role for epithelial-to-mesenchymal transition (EMT) and mesenchymal-epithelial transition (MET) during metastasis. The plastic state is likely to provide a significant advantage for migrating/invading epithelial cells because migrating cells need to reorganize their cytoskeleton and their vesicle trafficking and vesicle targeting machinery in order to transit from apical–basal polarity to front–rear polarity. Thus, changes in polarity proteins may lower the threshold for epithelial cells to acquire migratory/invasive cell behavior.

We had previously reported that mislocalization of Scribble phenocopies RNA interference (RNAi) knockdown of Scribble for cooperation with Myc during transformation of mammary epithelial cells. In our attempt to understand if mislocalization of Scribble is involved in cancer, we generated a mouse model overexpressing Scribble under the control of mouse mammary tumor virus (MMTV) promoter. We have discovered that transgenic mice expressing a Scribble mislocalization mutant display defects in mammary morphogenesis, followed by hyperplasia and tumor formation. Surprisingly, the tumors are pleomorphic and highly metastatic to the lung. We are pursuing these observations to understand how dysregulation of Scribble initiates metastatic tumors in mice.

Our studies continue to define a role for cell polarity proteins as regulators of cancer initiation and progression. A deeper understanding of the role played by cell polarity proteins is likely to identify a novel class of targets for controlling cancer.

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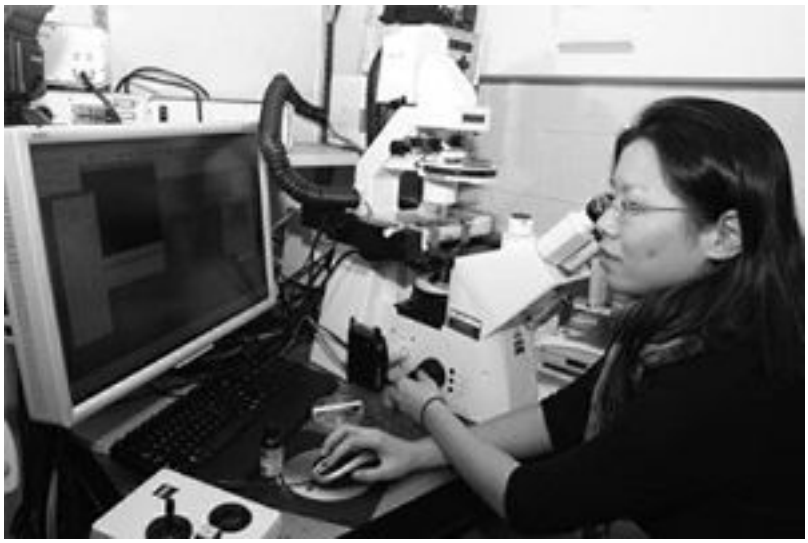

Bin Xue

PROTEOMICS LABORATORY AND PROTEOMICS SHARED RESOURCE

D. Pappin S. Bencze C.M. Gauss A. Lelo S. Peacock R. Rivera C. Ruse B. Weindorf J. Wilson

Proteomic Analysis of Proximal Pulmonary Venous Blood to Identify Biomarkers for Non-Small-Cell Lung Cancer

This work was done in collaboration with R. Sordella (CSHL) and B. Stiles and O. Elemento (Cornell University, New York).

Each year, more than 220,000 patients are diagnosed with lung cancer in the United States, with an overall 5-year survival rate of ≤15%. This is due to the advanced stage at diagnosis and to difficulties in detecting early tumors. As in the case of prostate-specific antigen (PSA) for prostate cancer, a reliable and easily accessible biomarker would prove invaluable in the early identification of patients with non-small-cell lung cancer (NSCLC). In fact, a recent randomized study highlighted the importance and impact of early diagnosis in NSCLC. Investigators from the National Lung Screening Trial reported that patients screened for NSCLC with computed tomography (CT) scans had a 20.3% decrease in mortality, exceeding the benefit of many therapeutic drug trials.

We are implementing a novel strategy to identify biomarkers from the serum of NSCLC patients by mass spectroscopy (MS) using iTRAQ (isobaric tagging for relative and absolute quantitation) technology. Unlike previous efforts aimed at identifying serum biomarkers using peripheral blood from NSCLC patients, the peripheral blood in our studies will be used only as reference, and blood draining directly from the tumor (pulmonary vein) will instead be used for biomarker identification. Because of its proximity to the cancer, the pulmonary venous blood (PVB) is highly enriched for tumor proteins, potentially allowing for greater sensitivity of detection of relevant proteins. In addition, each patient will serve as his/her own control, eliminating many of the sources of heterogeneity that have plagued the serum proteomic field and hampered biomarker discovery. The proteomic signature elaborated from the PVB will then be validated in the peripheral blood using more sensitive targeted techniques such as single- and multiple-reaction monitoring (MRM) and the clinical applicability tested in an independent set of blood from patients screened for lung cancer. We have already analyzed pulmonary venous blood, nontumor blood, and peripheral blood samples from four patients using the 8-plex iTRAQ reagent and 17-step multidimensional protein-identifcation technology (MudPIT) liquid chromatography–mass spectrometry (LC-MS). Preliminary data analysis is being performed now, and we anticipate collection of data from at least 30 additional patients over the next 12 months.

Proteomic Analysis of Cystic Fibrosis Sputum

This work was done in collaboration with S. Pattison (Queen's University, Belfast).

People with cystic fibrosis (CF) are unusually susceptible to lung infections, which contribute significantly to a poor prognosis. The majority of lung damage results from the abnormal, destructive action of the patient's immune system during times of extreme inflammation (known as pulmonary exacerbations). Although exacerbations are usually treatable with antibiotics, permanent lung damage frequently results. Accumulation of lung damage is responsible for the progressive decrease in lung function routinely associated with CF, a major detriment to patient quality of life and the most frequent cause of CF-related death. Exacerbations occur periodically, but their cause remains poorly understood. This research aimed to improve our knowledge of exacerbations and identify potential triggers. In particular, the study focuses on patients chronically colonized with *Pseudomonas aeruginosa*, because this is one of the most common and destructive infecting organisms.

In-depth proteomic and microbiological comparisons have already been made between sputum samples collected from 12 CF patients in times of relative good health versus pulmonary exacerbation, both before and after antibiotic treatment. This involved more than nine 8-plex iTRAQ MudPIT analyses during ~1 month of instrument time, and preliminary analysis of the collected data is now in progress. The proteomic analysis of patient sputum is of high clinical relevance because it effectively studies the interaction between host and

pathogens in vivo without introducing experimental bias through in vitro bacterial cultivation or the use of animal models. In addition, longitudinal sampling of the same patients allows documentation of changes in global protein expression in response to antibiotic treatment. Through the use of comparative proteomics to identify bacterial biomarkers linked to pulmonary exacerbation and disease pathogenesis, we hope to provide new targets for the development of improved preventative or therapeutic treatments to limit lung damage and enhance quality and length of life for CF patients.

Quantitation of Alternative Splice Variants at the Protein Level

This work was done in collaboration with M. Akerman, L. Zepeda, A. Krainer, and M. Zhang (CSHL).

The Shared Resource is working with the Krainer and Zhang laboratories to develop the use of iTRAQ quantitative proteomics to identify and quantify changes in exon-junction peptides as a measure of changes in alternative splicing isoforms of abundant proteins. In multiple duplex iTRAQ experiments, protein lysates were prepared from cytoplasmic and nuclear fractions of control HeLa cells and cells overexpressing the SF2/ASF splicing factor. The lysates were digested (trypsin) and labeled with two members of the 4-plex iTRAQ reagent set before mixing. The pooled, labeled peptides were analyzed by 15–16-salt-step MudPIT LC-MS or capillary LC-MS following first-dimension peptide separation by OffGel isoelectric focusing (24 fraction; pH 3–10). To quantify exon inclusion levels, we designed an exon isoform peptide database (PepDB). This captured all known transcripts encoded by every human gene using annotations from Ref-Seq and the expressed sequence tag (EST)-based alternative splicing database dbCASE. To account for every possible exon-skipping event, each transcript set was subdivided (i.e., encoded in a same gene) into exon trios by sliding a 3-exon window along the transcripts. The database was then formatted to allow exon-skipping quantification. By using this database as a mapping database for proteomic data, we can approach every human exon as an independent case and potentially estimate levels of alternative splicing.

Effect of Hypusination on the Tumor-Suppressive Function of eIF5a

This work was done in collaboration with S. Lowe (CSHL).

Tumor suppressor genes encode a broad class of molecules whose mutational attenuation contributes to malignant progression. In the canonical situation, the tumor suppressor is completely inactivated through a two-hit process involving a point mutation in one allele and chromosomal deletion of the other. To functionally identify tumor suppressor genes in lymphoma, the Lowe laboratory screened a short hairpin RNA (shRNA) library targeting genes deleted in human lymphomas to identify those genes whose suppression promotes tumorigenesis in a mouse lymphoma model. Among the new tumor suppressors they identified were adenosylmethionine decarboxylase 1 (AMD1) and eukaryotic translation initiation factor 5A (eIF5A), two genes associated with hypusine, a unique amino acid produced as a product of polyamine metabolism through a highly conserved pathway. Through a secondary screen surveying the impact of all polyamine enzymes on tumorigenesis, they established the polyamine-hypusine axis as a new tumor suppressor network regulating apoptosis. They next determined whether the expression of apoptotic regulators known to modulate Myc-induced lymphomagenesis (e.g., Bax, Bim, Puma, Bcl-2, Bcl-xL, and Mcl) were altered in premalignant B cells expressing shRNAs targeting the hypusination biosynthetic pathway. Bax was shown to be the only protein consistently underexpressed, and was also found to be reduced in a nonbiased iTRAQ-based proteomic analysis of premalignant B cells transduced with shRNAs targeting Eif5a and Amd1. Gene ontology analysis of the 431 differentially expressed proteins identified apoptosis as the most significantly enriched functional category. Precisely how Bax is controlled by Eif5a hypusination remains to be determined; nonetheless, Bax is a known eIF5A target and is an established suppressor of lymphomagenesis.

Posttranslational Modification of Papillomavirus Replication Factor E1

This work was done in collaboration with A. Stenlund (CSHL).

Papillomaviruses are responsible for a number of human diseases including genital papillomavirus infections and cervical cancer. The Stenlund laboratory has been investigating the biochemical properties of the viral E1 and E2 proteins, which are required for viral DNA replication. E1 is the initiator protein, which recognizes the origin of replication and also has DNA helicase activity. E2 regulates viral gene expression but is also involved in initiation of DNA replication. Recent studies have shown that phosphorylation of the E1 and

E2 proteins by the protein kinase CK2 regulates the activities of these proteins. We have analyzed the phosphorylation sites in the E1 protein after in vitro phosphorylation of recombinant E1 protein with recombinant CK2 enzyme. Briefly, the in-vitro-phosphorylated protein was digested with trypsin and with the nonsequence-specific protease elastase and subjected to MS analysis to identify phosphorylated peptides. In this manner, we were able to identify eight CK2 phosphorylation sites in the amino-terminus of the E1 protein. The modification of these sites has since been verified by mutational analysis.

Identification of Interacting Partners of LIN42

This work was done in collaboration with C. Hammell (CSHL).

Through forward genetics approaches directed toward understanding how aspects of developmental timing are controlled during animal development, the Hammell laboratory has identified the *Caenorhabditis elegans* homolog of the Period gene, *LIN42*, as a key player in the regulation of temporal gene expression. Through analysis of wild-type *LIN42* expression and phenotypes associated with *lin-42* mutants, they have determined that *LIN-42* functions to regulate the production and maturation of a large number of microRNAs, including those involved in development. In an effort to understand how *LIN-42* functions, we have complemented genetic approaches with a proteomic analysis of *LIN-42*-associated proteins via MudPIT. These efforts have identified a variety of chromatin-modulating components consistent with *LIN-42* functioning in the nucleus to control miRNA expression.

Posttranslational Modifications of the Splicing Factor SF2/ASF

This work was done in collaboration with Y. Liu, R.-Y. Tzeng, and A. Krainer (CSHL).

Continuing from earlier studies, we used a combination of bottom-up, middle-down, and multiple reaction monitoring (MRM) approaches to map posttranslational modifications of human SF2/ASF following magnetic bead immunoprecipitation of the protein from HeLa, A549, and 293E cells. For the bottom-up approach, a triple-digest protocol was used (trypsin, elastase, and subtilisin) followed by two-dimensional MudPIT LC-MS analysis of the overlapping, nonspecific digest products to increase coverage. For the middle-down approach, we have used Lys-C to generate larger fragments, which were analyzed by both collision-induced dissociated (CID) and electron transfer dissociation (ETD) fragmentation using the Orbitrap Velos mass spectrometer. In earlier studies, we identified six sites of SY phosphorylation, six sites of KR monomethylation, and one site of R dimethylation. We have since characterized the acetylation state of SRSF1. Previous discovery experiments had identified a few potential acetylation sites (e.g., K38 and K179). Immunoprecipitated SRP55 complexes from both HeLa and A549 cell lysates were subjected to triplicate MRM analysis, and we were able to confirm the presence of K(Ac)179-SRSF1 in both cell lines with almost 100% occupancy. Further work is in progress to uncover the biological function of these modifications.

Regulatory Modification of PTP1B by Persulfation

This work was done in collaboration with N. Krishnan, C. Fu, and N. Tonks (CSHL).

Protein tyrosine phosphatases (PTPs) have been established as regulators of a wide variety of signal transduction pathways, and they use an essential, reactive Cys residue. The Tonks laboratory tested whether H_2S may also target the catalytic Cys residue to regulate PTP function. Using high-resolution MS, we together identified a novel covalent modification (persulfation) of the active site Cys-215 in PTP1B that occurs in response to generation of hydrogen sulfide in vivo. This sulfhydration reaction also inactivates PTP1B, but it is subject to different mechanisms of reduction and reactivation compared to the reversibly oxidized enzyme. To understand the relevance of this modification to the control of cell signaling, the Tonks laboratory used RNA interference (RNAi) to suppress the critical enzyme in H_2S production, cystathionine-γ-lyase (CSE), in 293T cells, where they observed sulfhydration of Cys-215 following ER (endoplasmic reticulum) stress only in control cells. Using a novel phosphopeptide trapping mutant, we searched for potential substrates of PTP1B in the ER stress response, and identified a tryptic phosphopeptide derived from PERK (protein kinase-like ER kinase). The phosphorylation of Y619 in PERK has a critical role in the activation of PERK, which phosphorylates the eukaryotic translational initiation factor 2 (eIF2α), leading to translational atten-

uation. It has since been demonstrated that PTP1B dephosphorylates Y619 PERK and that this event is regulated by H_2S production in ER stress. These data suggest the possibility that different modes of reduction and reactivation of PTPs in response to distinct gasotransmitters may introduce new levels of control over signal transduction.

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John Wilson

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

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Lung cancer is one of the leading causes of death worldwide with mortality rates exceeding those of breast, prostate, and colon cancers combined. This is in part due to the lack of effective treatment and to the highly aggressive nature of non-small-cell lung carcinomas (NSCLCs), which account for the large majority of all cases of lung cancer. Thus, there was much excitement when treatments with erlotinib and gefitinib, two selective epidermal growth factor receptor (EGFR) inhibitors, resulted in dramatic regression of primary tumors as well as fewer metastases in patients with widely metastatic NSCLC. Remarkably, retrospective studies showed a striking correlation between occurrence of certain EGFR oncogenic mutations and erlotinib/gefitinib responses. Deletions in exon 19 of EGFR or EGFR L858R missense substitutions are found in more than 80% of NSCLC patients who respond to erlotinib or gefitinib treatment. Unfortunately, in the majority of cases, these responses are transient, and within a short period of time, patients who initially responded to treatment relapse. In ~50% of cases of erlotinib-acquired resistance, the acquisition of an additional mutation in exon 20 of EGFR resulting in a threonine-to-methionine substitution at position 790 (T790M) and/or amplification of c-Met accounted for treatment relapse. Thus, as is the case for other molecularly targeted therapies (e.g., imatimb, cetuximab, and tamoxifen), a better understanding of the molecular mechanisms responsible for resistance to gefitinib and erlotinib is crucial for effective clinical utilization of these drugs.

A TGF-β IL-6 Axis Mediates Selective and Adaptive Mechanisms of Resistance to Molecular-Targeted Therapy in Lung Cancer

To uncover new molecular mechanisms of gefitinib and erlotinib resistance in NSCLC, we developed a cell-based system. We cultured NSCLC-derived cell lines harboring EGFR oncogenic mutations (i.e., NCI-H1650 and HCC827 cells) in the presence of increasing erlotinib concentrations and isolated cells capable of growing in the presence of up to 30 μM of erlotinib. Among the erlotinib-resistant cell lines examined, we identified cell lines that had decreased erlotinib sensitivity but did not harbor the above-described mutations that led to erlotinib/gefitinib resistance. Interestingly, these erlotinib-resistant cells have morphological features of mesenchymal cells and have an increased motility, invasion, and metastatic potential. We refer to these cells as EMMs for erlotinib-resistant, mesenchymal, and metastatic cells. We found that activation of transforming growth factor-β (TGF-β)-mediated signaling was sufficient to induce these phenotypes. In particular, we determined that an increased TGF-β–dependent interleukin-6 (IL-6) secretion unleashed previously addicted lung tumor cells from their EGFR dependency.

Interestingly, gene expression profile analysis also revealed changes in the expression of the surface markers CD44 and CD24 (CD44 is high and CD24 is low in the resistant cells compared to the parental cells). By using these surface markers, we were able to show that the parental erlotinib-sensitive cells contained a subpopulation of cells (0.5%) that had features similar to those of EMM cells even before selection with erlotinib. Similarly, we identified EMM-like cells in human NSCLCs of different stages (Stages IA, IB, III) and in the bone marrow (BM) of patients. Importantly, a higher representation of EMM-like cells in early-stage lung tumors correlates with poor prognosis. Our data thus imply that erlotinib-resistant cells are already present in tumors before treatment and that metastatic spread and dissemination of tumor cells may be an early event leading to the eventual development of metastatic disease. This hypothesis departs from the long-held concept of development of resistance and metastasis as a consequence only of the acquisition of de novo mutations or late properties.

Because IL-6 and TGF-β are prominently produced during inflammatory response, we then used a mouse model system to determine whether inflammation might

impair erlotinib sensitivity. Indeed, induction of inflammation not only stimulated IL-6 secretion, but was also sufficient to decrease the tumor response to erlotinib.

This result argues that both tumor-cell-autonomous mechanisms and the activation of the tumor microenvironment could contribute to primary and acquired erlotinib resistance, and consequently, treatments based on EGFR inhibition may not be sufficient for the effective treatment of lung cancer patients harboring mutant EGFR.

Altogether, these studies highlighted potentially crucial aspects of tumor pathogenesis. Therefore, in the past year, we followed up these initial findings by actively pursuing a better understanding of the origin of EMM-like cells, their role in tumorigenesis, and the unique signaling pathways important for their survival.

An Epigenetic Switch Reprograms Metastasis-Initiating Cells and Their Pathways of Addiction through the Down-Regulation of miR-335

Because our initial observations were on in-vitro-based studies, we hypothesized that a cell-autonomous mechanism might account for the ontogeny of EMM-like cells. In collaboration with Dr. Ingrid Ibarra in Greg Hannon's lab at CSHL, we were able to show the presence of a distinct miRNA signature in the EMM-like cells compared to parental cells. Interestingly, we found that decreasing the expression of miR-335 in the parental cells was sufficient to induce phenotypic changes and a molecular signature characteristic of EMM-like cells.

miR-335 resides within the second intron of MEST/PEG1. As in the case of other intronic microRNAs (miRNAs), miR-335 expression has been associated with the expression of its host gene. Importantly, genetic deletion of miR-335 is a common event in human breast cancer, is enriched for in breast cancer metastases, and also correlates with ovarian cancer recurrence. Interestingly, in all of the cell lines examined and in NSCLC tumors, we did not find any deletion of the miR-335 locus; however, we did find a strong correlation between expression of miR-335, MEST, and methylation of a specific CpG island located in proximity to the MEST promoter region. Importantly, an overall decreased expression of MEST and miR-335 in early-stage NSCLC was associated with an increased probability of tumor relapse.

Among the direct targets of miR-335 identified by bioinformatics analysis and subsequent validation, we found genes involved in epithelial–mesenchymal transition (EMT), extracellular matrix remodeling, motility, osteoclast homeostasis, and self-renewal. Interestingly, miR-335 also regulated the expression of TGF-β indirectly by decreasing the expression of SRF, one of its direct targets. Interestingly, we found that TGF-β also regulates the expression of the "don't eat me" signaling molecule CD47. Our data thus suggest that an epigenetic switch is sufficient to reprogram tumor cells toward the acquisition of metastatics-initiating-cell-like features. This is of particular interest when considering that metastasis is a rate-limiting event. The ability to control this multistep process (e.g., extracellular remodeling, invasion, extravasation, survival in circulation, homing, and self-renewing) by the regulation of a single event increases the efficiency of metastatic spread and thus can explain early dissemination of tumor cells.

Yet miR-335 not only increased the metastatic potential of EMM-like cells, but also rewired their pathways of addiction. Although the parental cells are sensitive to phosphoinositol-3 kinase (PI3K) inhibition, both EMM-like cells and parental cells in which miR-335 was down-regulated are more resistant to PI3K inhibition and exquisitely sensitive to IL-6 axis inhibition. The fact that cells have become addicted to signaling mediated by IL-6 has important therapeutic implications. IL-6 neutralizing antibodies; JAK and STAT3 inhibitors have been developed by pharmaceutical companies and could be exploited to eliminate disseminating tumor cells (DTCs) and, in the context of erlotinb treatment, to overcome resistance. In addition, the association of increased sensitivity to JAK/IL-6 inhibition and a specific molecular signature could improve patients' stratification and can be used toward the development of tailored lung cancer therapies.

A Neomorphic p53 Isoform Involved in Injury-Repair Fails to Activate a Canonical p53 Tumor Suppressor Response but Confers Metastatic Features on Cells

Among the genes involved in DNA repair, gene expression profiling of EMM-like cells revealed a substantial decrease in expression of p53. The p53 tumor suppressor has a critical role in genome surveillance, suppression of oncogenic transformation, and regulation of many fundamental aspects of both reversible and irreversible cellular stress responses. p53 mutations, through loss of p53 function and putative gain-of-function activities, disable these effects and are associated

with aggressive cancers and poor patient prognosis. Although much is known about the underlying mechanisms of p53 regulation and activity, factors that influence the diversity and duration of p53 responses are not well understood.

When we measured p53 mRNA levels using reverse transcriptase–polymerase chain reaction (RT-PCR), we observed a less intense and slower migrating band specifically in the EMM cells, which we showed was the result of a novel alternative splicing event. The use of an alternative 3′ splice site in intron 6 generates a previously uncharacterized p53 isoform that lacks major portions of the DNA-binding domain, the nuclear-localization sequence, and the tetramerization domain. This p53 isoform, dubbed p53π, lacks transcriptional activity and fails to induce a canonical p53 tumor suppressor response. Yet, as is the case for certain gain-of-function p53 mutations, p53π expression is sufficient to decrease the expression of E-cadherin and to increase the invasive capability of cells. Importantly, this novel neomorphic p53 isoform appears to have a role in tissue-damage response by preventing premature senescence of cells involved in tissue remodeling and injury resolution in vivo. Our results thus identify a new mode of p53 regulation and suggest a physiological origin for gain-of-function activities of certain p53 mutants.

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Trine Linsted

PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes, including cell proliferation, differentiation, and survival. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects the coordinated activity of the kinases that phosphorylate it and the protein phosphatases that catalyze the dephosphorylation reaction. We study the family of protein tyrosine phosphatases (PTPs), which, like the kinases, comprise both transmembrane, receptor-linked forms as well as nontransmembrane, cytoplasmic species and represent a major family of signaling enzymes. Overall, the objective of the lab is to develop tools for analysis of PTP regulation and function and integrate them with state-of-the-art cell and animal models, to define critical tyrosine phosphorylation-dependent signaling events in human disease and thereby identify novel therapeutic targets. 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.

Having successfully defended their Ph.D.s, Guang Lin and Aftab Haque left the lab during the last year to take up postdoctoral positions with Hugo Bellen (Baylor) and Susan Lindqvist (MIT), respectively.

Novel Approaches to Therapeutic Inhibition of PTP1B

The protein tyrosine phosphatase PTP1B was the first member of this family of signaling enzymes to be purified, in work I conducted as a postdoctoral fellow. Interest in PTP1B from a therapeutic perspective has its foundation in diabetes and obesity, which along with the associated cardiovascular disease, pose a serious threat to public health in the 21st century. A recent report from WHO predicts that total prevalence of type 2 diabetes will rise from 171 million in 2000 to 366 million in 2030. The treatment options for diabetes and obesity largely remain unchanged since the 1940s, which implies a need for alternative, more effective ways to counter the disease. Our increased understanding of signal transduction in normal and pathophysiological conditions has suggested alternative therapeutic strategies in many major diseases that focus on manipulating signaling pathways. PTP1B plays a well-documented role as a negative regulator of insulin and leptin signaling. Targeted deletion of the *PTP1B* gene product in mice resulted in increased insulin sensitivity and resistance to obesity induced by a high-fat diet. These and other observations resulted in PTP1B being extensively validated as a suitable target for therapeutic intervention.

Apart from metabolic regulation, PTP1B is also implicated in breast tumorigenesis in response to HER2, the target for Herceptin. The *PTP1B* gene located at chromosome 20q13 is frequently amplified in breast cancer. In mice expressing HER2, a gene amplified in about 25% of breast cancer, loss of the *PTP1B* gene caused delayed tumorigenesis and the incidence of lung metastasis was decreased. These observations suggest that in addition to its role in attenuating insulin signaling, PTP1B *enhances* HER2-mediated signaling—it acts as a *positive* regulator of HER2 signaling to *promote* breast tumorigenesis. Therefore, inhibition of PTP1B would offer a novel avenue for therapeutic intervention in both diabetes and HER2-positive tumors, such as in breast cancer.

Several potent, small-molecule inhibitors of PTP1B have been developed; however, these inhibitors, which target the active site of the enzyme, are highly charged and consequently have poor bioavailability and limited drug development potential. Therefore, there is a pressing need for an alternative approach to generate inhibitors against PTP1B that do not involve the active site of the protein.

We have identified a novel allosteric inhibitor of PTP1B that binds to the noncatalytic portion of PTP1B and does not depend on charge for interaction. Several biochemical tools were used to define a novel mechanism for inhibition of PTP1B by this molecule. Therefore, we were interested in testing the inhibitor in breast cancer models where PTP1B may play a signaling function.

We have succeeded in demonstrating that this PTP1B inhibitor antagonized HER2-induced cell migration in MCF10A mammary epithelial cells that express a well-characterized, inducible chimeric form of HER2. We have tested analogs of the molecule and observed that the effects on migration correlated with the potency of PTP1B inhibition. Furthermore, we tested the inhibitor in multiple cell lines and observed that it antagonized migration preferentially in HER2-positive cells. In addition, we have incorporated data from a three-dimensional (3D) culture system that recapitulates several aspects of mammary gland architecture in vivo and has been used as a model to study the biological activities of cancer genes. In this system, activation of HER2 results in the formation of hyperplastic structures in 3D culture; this was attenuated by treatment of the cells with the PTP1B inhibitor.

The most recent results are the most exciting. We have tested the inhibitor in a transgenic mouse model, the NDL2 mouse, which expresses an activated mutant form of HER2 and develops mammary tumors and lung metastases that display features of human breast cancer. In two independent experiments, we allowed these transgenic mice to develop palpable tumors before treating with the inhibitor. Treatment resulted in marked reduction of tumor growth. Perhaps most importantly, we observed that treatment with the inhibitor of PTP1B was accompanied by a dramatic attenuation of metastasis to the lungs. These data reinforce the therapeutic potential of inhibiting PTP1B by this allosteric mechanism to antagonize HER2-induced signaling and tumorigenesis. Currently, we are working with scientists at SWOG (one of the leading National Cancer Institute–supported cooperative groups) to complete a preclinical data package and to pursue the testing of this molecule in clinical trials for HER2-positive breast cancer. Ultimately, we plan to test this inhibitor and derivatives of it in models of diet-induced obesity and insulin resistance.

Characterization of Novel Cancer Therapeutics

Avemar® is a proprietary fermented wheat germ extract (FWGE) that has been shown to have many beneficial characteristics, including potent anticancer, anti-inflammatory, immune-modulatory, metabolic-regulatory, cardiovascular-protective, and anti-aging properties. FWGE impairs critical aspects of the transformed phenotype, including aerobic glycolysis, the pentose phosphate pathway, and ribonucleotide reductase. It displays significant antiproliferative effects and triggers tumor cell death through apoptosis. Clinical data reveal significant benefits to patients from treatment with FWGE, including in combination with existing cancer therapies. FWGE likely comprises thousands of different molecules. In particular, it is known to contain two biologically active compounds: lectins (WGA, wheat germ agglutinin) and methoxy-substituted benzoquinones (DMBQ, 2,6-dimethoxy-*p*-benzoquinone; MBQ, 2-methoxybenzoquinone). Nevertheless, neither of these components are the physiologically significant active molecules of the extract. Although current data support the use of FWGE as a nonprescription nutraceutical in various cancers, it is clear that exploiting its full potential will require a more precise definition of the active ingredient core components of the mixture and the biochemical characterization of their mechanism of action. During the past couple of years, we have made considerable progress in purifying the active components of Avemar®, using procedures that are readily adaptable to large-scale production. Various fractions from the initial purification procedure have been screened in cell viability assays on a broad panel of tumor cell lines and animal models, with encouraging results. In an important first step, a fraction has been generated, termed A250, which represents ~3% of the dry weight of Avemar®, yet retains essentially all of the activity, and has the potential to represent a new product. In collaboration with Darryl Pappin and Jim Watson here at CSHL and Mate Hidvegi and his colleagues from Budapest, we are now focusing on producing stable, enriched, and well-characterized fraction(s) from crude FWGE that show significant activity in cell and animal models of cancer, with the goal of developing novel therapeutics.

Other Projects Brought to Successful Publications

We published the results of a comprehensive, PTP family-wide RNA interference (RNAi) screen to test systematically the role of PTPs in controlling migration and invasion in mammary epithelial cell models of breast cancer. The analysis revealed many novel insights, including

the discovery that the phosphatase PTPN23 is a regulator of mammary cell invasion that exerts its effects via regulation of the protein tyrosine kinase SRC. One of the important conclusions of this study is that inhibitors of SRC may offer a novel therapeutic approach to treating patients with inactivating mutations in PTPN23, the gene for which is located at chromosome 3p21 in a hot spot mutated in breast and other cancers. This is now being tested in animal models. In addition, we have completed a loss-of-function screen for the effects of the classical PTPs on growth of MCF10A cells in 3D culture. This has identified several novel roles for specific PTPs in the regulation of HER2 signaling, the mechanistic basis for which are now being pursued.

We published a major paper describing the generation of conformation-sensor antibodies that recognize selectively the oxidized form of PTP1B and the demonstration that, when expressed in cells, they lead to enhanced and sustained insulin signaling. This is important because it suggests a new paradigm for PTP-directed drug development, particularly for treatment of diabetes and obesity.

We published a paper describing the regulation of PTPs by "gasotransmitters," which are a class of gaseous signaling molecules that are freely permeable to membranes and, therefore, unlike classical regulators of signal transduction, they act independently of transmembrane receptors. Hydrogen sulfide (H_2S) has long been considered a toxic molecule; however, with the burgeoning interest in gasotransmitter function, it has recently been implicated in the regulation of several biological processes. A major gap in our understanding of the physiological function of H_2S, and its potential role in signaling, is the identity of its cellular targets. We examined whether H_2S regulates PTP function and revealed a novel tier of control of the unfolded protein response (UPR) to endoplasmic reticulum (ER) stress.

The ER plays a major role in controlling the folding and modification of newly synthesized transmembrane and secreted proteins. The folding capacity of the ER is modulated in response to the environmental and physiological status of the cell; thus, a cell can match its capacity for protein folding to its physiological requirements. However, if that capacity is exceeded, resulting in a condition known as ER stress, an integrated set of three signaling pathways, the UPR, is activated. These pathways are triggered by transmembrane sensor proteins that detect unfolded proteins in the ER and transduce a signal that initiates a response in the cytosol or nucleus. This allows the cell to fine-tune the rates of protein synthesis and its folding capacity to maintain homeostasis and, if that is not successful, to trigger cell death. We demonstrated that ER stress leads to the production of H_2S, which generates a persulfide modification of the active site Cys of PTP1B, with concomitant inhibition of phosphatase activity. This, in turn, controls the ability of PTP1B to dephosphorylate PERK (protein kinase-like ER kinase), which is one of the transmembrane sensors of unfolded proteins (Fig. 1). PERK normally phosphorylates the eukaryotic translational initiation factor 2 (eIF2α) leading to translational attenuation, which limits the load of unfolded protein entering the ER. Thus, the regulation of PTP1B by H_2S is a critical step in controlling the activity of PERK and, thereby, the ability of a cell to monitor and regulate its protein folding capacity. This work introduces a new level of control over signal transduction, which may provide fundamentally important insights into the etiology of diseases associated with aberrant protein folding, such as Alzheimer's and Parkinson's.

Using an organotypic brain slice culture system, in conjunction with 2-photon imaging, we have produced a system in which to examine PTEN function in neurons. Consistent with the increase in spine density that has been shown to accompany the loss of PTEN in knockout mouse models, we observed that expression of wild-type green fluorescent protein (GFP)-PTEN resulted in a dramatic decrease in spine density compared to control neurons transfected either with GFP alone or

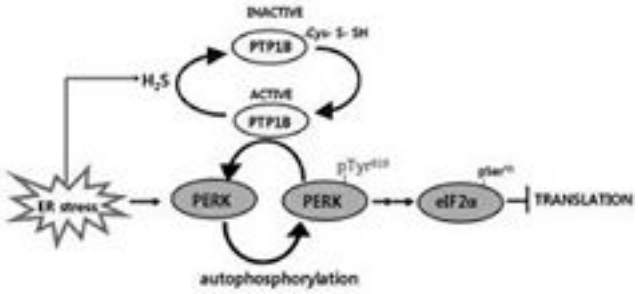

Figure 1. Proposed model for the role of sulfhydration of PTP1B in regulating the response to ER stress. Induction of ER stress increases the production of H_2S by cystathionine-γ-lyase, which leads to sulfhydration of the essential active site cysteine residue in PTP1B and concomitant inactivation of the phosphatase. This transiently protects pTyr 619 of PERK from dephosphorylation, promoting PERK activation and its ability to inhibit global translation by phosphorylating eIF2α. Reduction and reactivation of sulfhydrated PTP1B restores phosphatase activity and its ability to dephosphorylate and inactivate PERK. (From Krishnan et al. 2011.)

with inactive mutants, thus permitting us to use the density of dendritic spines as a biological read-out of PTEN function. In vitro, the tumor suppressor PTEN displays intrinsic phosphatase activity toward both protein and lipid substrates. In vivo, the lipid phosphatase activity of PTEN, through which it dephosphorylates the 3 position in the inositol sugar of phosphatidylinositol derivatives, is important for its tumor suppressor function; however, the significance of its protein phosphatase activity remains unclear and represents a gap in our understanding of the function of this important regulator of cell signaling. Using this bioassay, we have demonstrated that it was the protein phosphatase activity, but not the lipid phosphatase activity, that was essential for the ability of PTEN to decrease neuronal spine density. Furthermore, these effects of PTEN depended upon the phosphorylation status of Ser and Thr residues in its carboxy-terminal segment and the integrity of the carboxy-terminal PDZ-binding motif. Overall, our data suggest that PTEN autodephosphorylation may be a critical event in this process, exerting a direct influence on its interaction with PDZ domain-containing proteins. The data reveal that in addition to dephosphorylating the 3 position in phosphatidylinositol phospholipids, the critical protein substrate of PTEN may be PTEN itself. Thus, we demonstrate a striking parallel with phosphatidylinositol-3 kinase (PI3K), which phosphorylates exogenous lipid substrates and displays protein kinase activity through autophosphorylation, illustrating symmetry in the regulation of signaling via phosphorylation and dephosphorylation of inositol phospholipids.

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EMERGING PRINCIPLES OF TUMOR SUPPRESSION

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PTEN is a tumor suppressor that is among the most frequently lost or mutated genes of human cancer. It is unique in directly opposing the enzymatic activity of phosphoinositol-3 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, the discovery of the senescence response suggests that tumors should favor retention of some PTEN. Indeed, many human cancers present with either 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 enabling us to probe for genome-wide changes that are consistent with the progression features identified in mouse. Conversely, we also probe 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.

From Indolent Prostate Cancer to Lethal Disease

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[in collaboration with A. Newton, University of San Diego, California; B. Carver and C. Sawyers, Memorial Sloan-Kettering Cancer Center, New York]

Understanding the progression from indolent to lethal prostate cancer remains the major challenge in the field. Hyperactivation of the PI3K/AKT pathway is common in many cancer types. Tumorigenesis through this pathway is prevented by concerted action of multiple tumor suppressor genes. Most notably, PTEN reverts PI3K activity, whereas excessive pathway activation triggers p53-mediated senescence arrest. However, it has remained unclear if and at what stage this response acts in human prostate cancer.

We have identified the AKT-inactivating phosphatase PHLPP1 as a tumor suppressor and demonstrated how the senescence response antagonizes codeletion of *Pten* and *Phlpp1* genes to form a barrier against prostate cancer progression in mouse. We could show that *Phlpp1* loss causes neoplasia and, upon partial *Pten* loss, carcinoma in mouse prostate. This genetic setting triggered growth arrest via protein translation-dependent activation of p53. Thus, we found that tumor formation in the mouse required spontaneous inactivation of the *p53* gene. Validating this conditional gene inactivation scheme in a comprehensive genomic patient data set, we found that codeletion of *PTEN* and *PHLPP1* is almost exclusively observed in metastatic prostate cancer and highly correlated to deletion of *TP53*. Furthermore, we found that *PTEN/PHLPP1* mRNA expression can be used for outcome prediction in this patient cohort, thus adding actionable molecular information on pathway status to the standard histology-based Gleason method.

We showed that the Phlpp2 protein compensates for *Pten* loss in a pathway feedback, which rationalizes their codeletion with *PTEN* in late-stage prostate cancer. This important feedback response is blocked by pharmacological pathway inhibition that inhibits mTORC1. Thus, mTORC1-mediated translation is important for supporting high levels of p53 and PHLPP2 when *PTEN* is lost. Our finding that these three genes are genetically abolished only in the lethal disease emphasizes the importance of the feedback response. Together, our findings can help the development of improved molecular tools for prostate cancer prognosis and therapy and highlight the value of our genetically engineered mouse models for the process.

In the Brain: PTEN and Stroke

A. Naguib [in collaboration with S.-S. Tan and J. Howitt, The University of Melbourne, Australia]

PTEN is the major negative regulator of PI3K signaling with cell-specific functions that go beyond tumor suppression. We have previously shown that nuclear import of PTEN is essential for tumor suppression. Yet, it has remained unclear what physiological stimulus determines the variety of nucleo-cytoplasmic distributions of PTEN that are observed in tissues. In collaboration with experts in mouse models for stroke, we have demonstrated that cytoplasmic Pten is translocated into the nucleus of neurons following cerebral ischemia. Critically, this transport event is dependent on the surge in the Ndfip1 protein, as neurons in *Ndfip1*-deficient mice fail to import Pten. Ndfip1 binds Pten, resulting in enhanced ubiquitination by Nedd4 E3 ligases, an event that we previously showed to control import. In vitro, Ndfip1 overexpression increases the rate of Pten nuclear import detected by photobleaching experiments, whereas *Ndfip1*$^{-/-}$ fibroblasts show very poor import rates. In vivo, *Ndfip1*-mutant mice suffer larger infarct sizes associated with suppressed pAkt activation. These findings have unraveled the first physiological stimulus to trigger shuttling of Pten into nuclei. Moreover, they suggest that getting PTEN out of the cytoplasm and into the nucleus for a limited time after stroke is critical for neuronal survival and prevents irreversible brain damage. These findings may thus open novel avenues for therapeutic intervention after stroke, centered around ensuring proper trafficking of PTEN.

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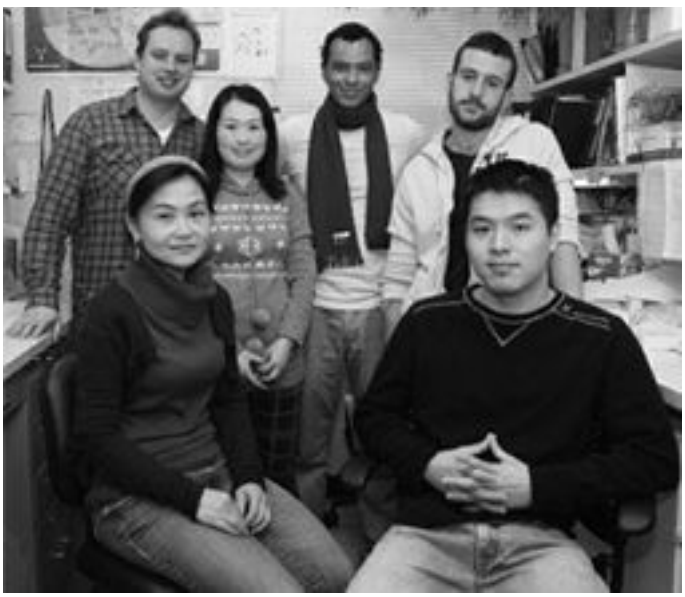

Lloyd Trotman (*center, back*) and lab members

RAS AND RHO GTPases AND THE CONTROL OF SIGNAL TRANSDUCTION

L. Van Aelst B. Boettner, X.L. Gu, J. Janas, A. Kobayashi, Y. Tai, C.-L. Wang, Y.-T. Yang, J.-R. Yu

Research in my laboratory focuses on signal transduction pathways involving Ras and Rho GTPases. Ras and Rho family members have key roles in cellular activities controlling cell growth control, differentiation, and morphogenesis. Alterations in Ras and Rho functions have been shown to result in 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 processes. Toward this end, my lab has continued to define the functions of selected GTPases, their regulators, and effectors in models of cancer and neurodevelopmental disorders. Below are highlighted the main projects that have been carried out during the past year.

Rapid Synthesis of the X-Linked Mental Retardation Protein OPHN1 Mediates mGluR-Dependent LTD through Interaction with the Endocytic Machinery

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 have begun to unveil the function of OPHN1 at both the presynaptic site and the postsynaptic site of the hippocampal CA3-CA1 synapse. We previously showed that during early development, presynaptic OPHN1 is important for efficient retrieval of synaptic vesicles, whereas postsynaptic OPHN1 has a key role in activity-dependent maturation and plasticity of excitatory synapses, suggesting the involvement of OPHN1 in normal activity-driven glutamatergic synapse development. We have now found that postsynaptic OPHN1 also has a critical role in mediating a form of plasticity (mGluR-LTD [long-term depression]) that relies on the activation of group I metabotropic glutamate receptors, which consist of mGluR1 and mGluR5 in CA1 hippocampal neurons. Alterations in this form of plasticity have been linked to drug addiction and cognitive disorders. A key characteristic of mGluR-LTD is its dependence on rapid protein synthesis; however, the identities of the proteins mediating LTD have remained largely elusive. We obtained evidence that OPHN1 expression is translationally induced in dendrites of CA1 neurons within 10 min of mGluR activation and that this response is essential for mGluR-dependent LTD. Specifically, acute blockade of new OPHN1 synthesis impedes mGluR-LTD and the associated long-term decreases in surface AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors). Interestingly, the rapid induction of OPHN1 expression is primarily dependent on mGluR1 activation and is independent of fragile X mental retardation protein (FMRP). Importantly, we further demonstrated that OPHN1's role in mediating mGluR-LTD is dissociable from its role in basal synaptic transmission. Regulation of basal synaptic strength requires OPHN1's Rho-GAP activity and association with Homer 1b/c proteins, whereas mGluR-LTD and the associated long-term decreases in surface AMPARs are dependent on OPHN1's interaction with endophilin A2/3. Together, these findings point to a multifunctional role for OPHN1 at CA1 synapses. Independent of its role in activity-driven glutamatergic synapse development, regulated OPHN1 synthesis has a critical role in mGluR-dependent LTD. Thus, it is conceivable that, on the one hand, OPHN1 might have an important role in synapse maturation and circuit wiring dur-

ing early development; on the other hand, the regulated OPHN1 synthesis could operate during adulthood to weaken synapses in response to behaviorally relevant stimuli.

DOCK180 Family Member DOCK7 Controls Multiple Steps of Neuronal Development

DOCK7 is a member of the evolutionarily conserved DOCK180-related protein superfamily, which we initially identified as a novel activator of Rac GTPases. We found that DOCK7 is highly expressed in major regions of the brain, including hippocampus and cortex, 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 for a key role of DOCK7 in controlling the early steps of axon development in cultured hippocampal neurons. Knockdown of DOCK7 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 uncovered a distinct role for DOCK7 in the regulation of cortical neurogenesis. Neurogenesis in the developing neocortex relies on the ability of radial glial progenitor cells (RGCs) to switch from proliferative to differentiative neuron-generating divisions, but the molecular mechanisms that control this switch in a correct temporal manner are not well understood. We found that DOCK7 has a key role in the regulation of RGC proliferation versus differentiation. In particular, silencing of DOCK7 in RGCs of developing mouse embryos impedes neuronal differentiation and maintains cells as cycling progenitors. In contrast, DOCK7 overexpression promotes RGC differentiation to basal progenitors and neurons. We further obtained evidence that DOCK7 influences the mode of RGC division and thereby neurogenesis by controlling apically directed interkinetic nuclear migration (INM) of RGCs. Importantly, DOCK7 exerts these effects by antagonizing the microtubule growth-promoting function of the centrosome-associated protein TACC3. Thus, DOCK7 interacts with TACC3 to control INM, thereby governing RGC proliferation versus differentiation and genesis of neurons during cortical development—processes associated with numerous neurodevelopmental disorders.

Oncogenic Tyrosine Kinases Target Dok-1 for Ubiquitin-Mediated Proteasomal Degradation to Promote Cell Transformation

Dok-1 (also called $p62^{dok}$) was initially identified as a tyrosine phosphorylated 62-kDa protein associated with Ras-GAP in Ph^+ chronic myeloid leukemia (CML) blasts and in v-Abl-transformed B cells. This protein was termed Dok (downstream of kinases), since it was also found to be a common substrate of many receptor and cytoplasmic tyrosine kinases. Subsequently, six additional Dok family members have been identified, Dok-1 to Dok-7. Among them, Dok-1 and Dok-2 share the ability to bind to a negative regulator of Ras, Ras-GAP. We previously showed 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 $p210^{bcr\text{-}abl}$, suggesting that it possesses tumor suppressive activity in the context of myeloid leukemia. In support of this, we found in collaboration with Dr. Pandolfi's group that mice lacking both *Dok-1* and *Dok-2* spontaneously develop a CML-like myeloproliferative disease. Furthermore, more recent studies have shown that mice with combined Dok-1, Dok-2, and Dok-3 knockouts also develop lung adenocarcinoma with penetrance and latency dependent on the number of lost Dok family members.

These studies thus indicate that the Dok-1 to Dok-3 proteins possess tumor suppressive activities and that their inactivation can contribute to disease/tumor progression associated with deregulated protein kinase signaling, as, for example, in the case of $p210^{bcr\text{-}abl}$-driven CML-like disease in mice. To date, however, very little is known about the regulation of Dok proteins by oncogenic tyrosine kinases (OTKs). We have focused on Dok-1 and mechanisms of its regulation by $p210^{bcr\text{-}abl}$ and other OTKs. We found that OTKs, including $p210^{bcr\text{-}abl}$ and oncogenic forms of Src, down-regulate Dok-1 by targeting it for degradation through the ubiquitin-proteasome pathway. This process is dependent on the tyrosine kinase activity of the oncoproteins and

is mediated primarily by lysine-dependent polyubiquitination of Dok-1. Importantly, restoration of Dok-1 levels strongly suppresses transformation of cells expressing OTKs, and this suppression is more pronounced in the context of a Dok-1 mutant that is largely refractory to OTK-induced degradation. Together, our findings indicate that proteasome-mediated down-regulation of Dok-1 is a key mechanism by which OTKs overcome the inhibitory effect of Dok-1 on cellular transformation and tumor progression.

New Functions of Rap1 Signaling in Cell–Cell Adhesion and Epithelial Morphogenesis

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 Rap1 acts independently of Ras signaling. Rap1 function is critical for cell adhesion processes during tissue morphogenesis, and recent studies suggest that dysregulated Rap1 signaling may contribute to malignant manifestations. However, how Rap1 controls cell adhesion in vivo has remained largely elusive. Using genetic tools and cell-based interaction assays, we have identified a signaling module that contains PDZ-GEF, a Rap1-specific exchange factor, Rap1 itself, and the adaptor protein AF-6. Our study of this module in *Drosophila* demonstrated that productive signal transduction through DPDZ-GEF, Rap1, and the *Drosophila* AF-6 ortholog Canoe governs epithelial migration and gastrulation events in the embryo at mitotically silent stages. 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 in close association with cell–cell adhesion complexes.

Following up on these observations, we identified an additional role for Canoe/AF-6 as a Rap1 effector in mitotically active epithelia, such as the highly proliferative wing imaginal disc. Our experiments showed that AF-6/Canoe is key to a mechanism that reconstitutes E-cadherin-based adherens junctions (AJs) at the apical membrane between sister cells, late in cytokinesis, in each cell division event. *Canoe* mutant cell populations display a dramatic loss of cohesion, do not sort to their proper positions within the epithelium, and become invasive. We searched for genetic modifiers of the partial AJ loss between *canoe* mutant cells and discovered a critical synergism with the Echinoid protein, the *Drosophila* homolog of the Nectin family of IgG adhesion proteins. Nectins in mammalian cells were proposed to function by directly interacting with AF-6 in cultured epithelial cells. Our results, however, support a model in which Rap1 and Canoe act independently of, but in synergy with, Echinoid to control the overall homeostasis of AJs. Importantly, the general and almost complete inability to form AJs that we observe in cell populations lacking both Echinoid and Canoe is coupled to a partial breakdown of the apico-basal polarity machinery. The strong synergism between Echinoid and Canoe therefore unravels a molecular link between polarity systems and the establishment of normal AJs. In light of the conservation of the structure and function of AJs and the polarity machinery between *Drosophila* and mammalian epithelia, our findings have important implications for mammalian epithelial tissue morphogenesis in vivo.

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REGULATION OF MALIGNANT GLIOMA HETEROGENEITY AND LINEAGE DEVELOPMENT

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Malignant glioma represents the most common and lethal type of brain tumor and is stratified into distinct pathologic grades with well-known biological behaviors. Clinically, although WHO (World Health Organization) grade I astrocytomas exhibit defined margins and may be removed surgically, higher-grade tumors often disseminate and invade into surrounding brain parenchyma. The presence of tumor extensions beyond their visually identifiable borders precludes complete surgical resection. As a result, postsurgery tumor recurrence and/or progression to a higher grade are inexorable. In its most severe manifestation, the glio- blastoma multiforme (GBM), the median survival is only ~12–15 months after initial diagnosis with a 5-year survival rate <3% despite abundant treatment efforts. Unfortunately, in the last several decades, refinements of available therapeutic modalities including microneurosurgery, radiotherapy, and chemotherapy have so far failed to substantially improve survival. Thus, new targeted therapeutic approaches based on a deeper understanding of glioma biology are urgently needed. Along this line, we have directed our research around two major areas: (1) We are developing various animal models to recapitulate human glioma pathogenesis and are utilizing them as tools to trace and investigate the in vivo disease initiation/progression, and (2) we are applying integrated approaches combining mouse models, stem cell biology, and RNA interference (RNAi) to identify the molecular and developmental pathways relevant to glioma pathogenesis and treatment. We are particularly interested in genetic programs and pathways governing normal and glioma cell lineage development with an emphasis on the intimate relationships between cellular renewal and differentiation. We believe that an improved understanding of the developmental pathways governing the self-renewal and differentiation processes along the neural progenitor–glial axis and, by extension, the glioma initiation cells–progeny axis will be instrumental in guiding future development of efficient treatments targeting this dreadful disease.

Characterization of EGFR-Targeted Therapeutic Resistance

As a signature genetic event of malignant gliomas, the epidermal growth factor receptor (EGFR) amplification/mutation is present in ~40%–50% of human patient samples, making it a compelling candidate for targeting therapy. Although countless studies have repeatedly demonstrated the crucial roles of aberrant EGFR signaling in glioma cell growth and survival in vitro, unexpectedly, the promising laboratory experimental results seen with EGFR inhibitors have thus far not translated into clinical success. Results from multiple clinical trials using different EGFR-specific inhibitors or antibodies indicated that the majority of patients whose tumors express amplified EGFR and who would be expected to respond, do not. Even in those patients who did show some clinical or radiological response to EGFR inhibitors, the benefit was not durable and the disease progressed rapidly thereafter. Clinically, this quick acquisition of resistance to anti-EGFR drugs poses a huge obstacle to successful therapy. However, due to absence of appropriate biological systems to interrogate in vivo disease progression, the mechanism(s) underlying the EGFR-targeted therapeutic resistance remains unclear.

Against this backdrop, we have recently generated a novel tetracycline-inducible glioma mouse model in which overexpression of a malignant glioma-specific mutant EGFR is engineered with concurrent brain-specific inactivation of *Ink4a/Arf* and *Pten*, recapitulating the genetic abnormality observed in the human patient samples. Importantly, the tightly tetracycline-controlled mutant EGFR expression in this case ensures a faithful model system to mirror EGFR-targeting therapy, therefore providing us an opportunity to dissect the resistance mechanism(s) under in vivo settings. Phenotypic analysis revealed that the compound mice with mutant EGFR induction developed malignant gliomas with a median latency of about 35 weeks. Remarkably, although tumor initiation in these mice was strictly dependent on induced mutant EGFR expression, we found that after a

short initial response upon doxycycline or EGFR-inhibitor treatment, the developed gliomas were able to rapidly acquire resistance to EGFR inhibition. In addition, the resistant tumors continued to progress independent of aberrant EGFR signaling, a situation essentially mirroring the EGFR-targeted therapeutic resistance observed in human patients.

EGFR gene amplification in human malignant glioma occurs invariably as extrachromosomal double-minute fragments. When devoid of selective pressure, the amplified *EGFR* diminishes rapidly upon cycles of cell division. As a result, heterogeneous intratumoral *EGFR* amplification and gene expression are commonly observed in human malignant gliomas. This heterogeneous EGFR expression pattern is also notably recapitulated in our inducible EGFR mouse gliomas, often with the high EGFR expression cells present in the peripheral invasive edge of the tumors compared to low EGFR expression subpopulations in the relatively solid tumor center. Although aberrant EGFR expression is essential for glioma initiation in this mouse model, the existence of large subpopulations of low EGFR expression tumor cells in the developed tumors nevertheless suggests to us that aberrant EGFR signaling is not absolutely required for all tumor cell survival. And these subpopulations of low EGFR expression tumor cells may propagate independent of aberrant EGFR signaling and therefore be inherently resistant to EGFR target therapy. Along this line, our serial in vivo experiments further indicated that the EGFR-targeted therapeutic resistance in malignant glioma is indeed largely attributable to the inherent intratumoral heterogeneity and likely sustained by the preexisting tumor cell subpopulation(s) that engages alternative survival signaling(s) independent of the EGFR pathway. We are currently applying an integrated approach to investigate the critical signaling modules that persist within the resistant glioma cells. By identifying the potential resistance mechanism(s), we hope to provide the rationale for the future design of new combinational therapeutic strategies targeting this dreadful disease.

Epigenetic Regulation during Glioma Pathogenesis

Malignant glioma is a genetic disease driven by the accumulation of numerous genetic and genomic alterations. At the same time, it is also a highly complex developmental disease. A single tumor can act as an evolving biological system containing a vast heterogeneous cell population, reminiscent of normal tissue renewal with neural stem/progenitor cells (NPCs) at the apex of the hierarchy. Pathologically, malignant glioma is well known for its explicit intratumoral heterogeneity. But unlike the normal NPCs, the glioma cells are largely devoid of terminal differentiation traits and possess aberrant proliferative capacity. The recent identification of tumor-initiating cells (TICs) with stem-cell-like properties in malignant glioma underscores this developmental plasticity and suggests targeting glioma cell lineage differentiation as a promising avenue for developing effective therapeutics. Previously, we had established a malignant glioma model driven by the brain-specific inactivation of *p53* and *Pten*, the two most common mutational events found in human GBM. On the basis of this mouse model, we were able to reproducibly isolate and culture the primary glioma cells enriched for glioma TICs. These mouse glioma TICs, unlike the normal NPCs, were resistant to differentiation induction. Importantly, we found forced restoration of the differentiation capacity within these tumor cells could largely compromise their gliomagenic potential, making them a great primary cell-based system to study glioma TIC differentiation. Epigenetic regulation as the core mechanism has been implicated in controlling cellular fate determination, and thus, in collaboration with Scott Lowe and Chris Vakoc's groups at CSHL, we set out to identify the epigenetic networks essential for maintaining glioma TIC against differentiation. We screened a customized RNAi library targeting 243 known chromatin regulators against the mouse glioma TICs developed from our model systems. The primary short hairpin RNA (shRNA) screen was performed using a one-by-one approach in three independent glioma TIC lines. Right now, we have finished about three-quarters of the primary screen in which a total of 78 shRNAs targeting 32 candidate genes were identified. We anticipate that we will be able to finish the primary screen within the near future. By using a stringently filtered list of candidates and rigorous functional validation systems, we hope that this project will elaborate a spectrum of prime targets that can be enlisted into productive drug discovery and development efforts against malignant gliomas.

Conversely, we have also been probing the same epigenetic RNAi library for chromatin modulators whose suppression may compromise the neural progenitor cell's differentiation capacity and therefore act as tumor suppressor genes to prevent malignant transformation. We have identified several interesting candidates and are currently investigating their mechanistic functions in glioma initiation and progression.

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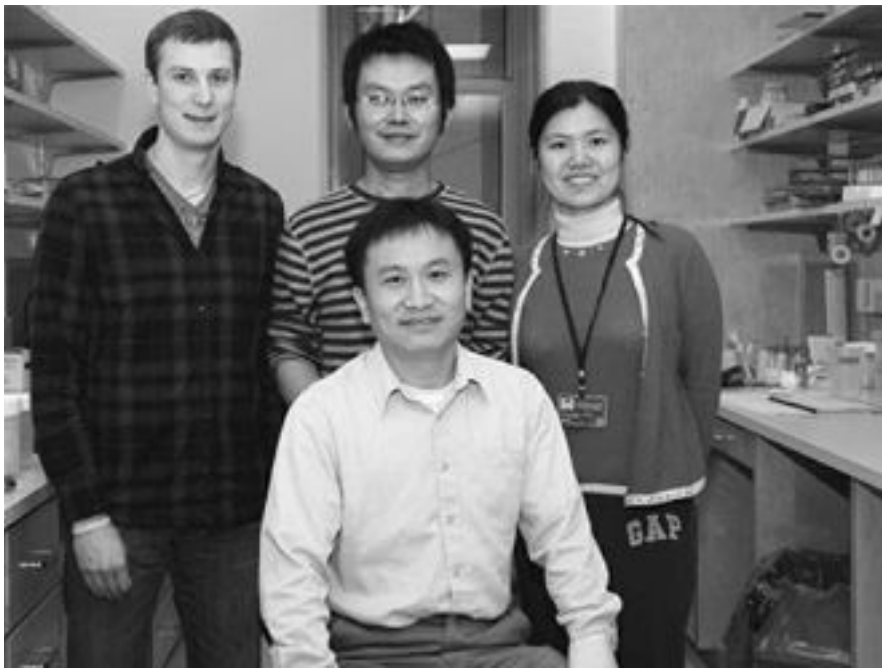

(*Left to right*) Stefan Klingler, Baofeng Guo, Ling Zhang, Hongwu Zheng (*seated*)

NEUROSCIENCE

CSHL neuroscientists focus on understanding how neural activity and neural circuitry underlie behavior, and how disruptions in these circuits lead to neurological and neuropsychiatric disorders such as Alzheimer's disease, autism, schizophrenia, and depression. These questions are addressed in two model systems—rodents and *Drosophila*—using molecular, cellular, genetic, developmental, theoretical, physiological, and behavioral approaches. Neuroscience research at CSHL is highly collaborative, and it can be divided into three broad themes: sensory processing, cognition, and cognitive disorders. In addition, there is an effort to develop new anatomical methods to improve our understanding of brain circuits, connectivity, and function. How does the brain encode stimuli from the outside world, within and across sensory modalities, to generate specific perceptions that, in turn, trigger complex behaviors? How is the brain shaped by sensory experience and what modifications occur in neuronal circuits that allow us to learn and remember? These are questions guiding the work of Florin Albeanu, who is using the olfactory bulb and olfactory cortex of mice as the subject of his current studies. Airborne chemicals, translated into neuronal signals by specific receptors in the nose, are sent directly to the olfactory bulb. Advances in optical imaging and optogenetics, combined with electrophysiological recordings, enable Albeanu and colleagues to monitor and/or alter patterns of activity at unprecedented synaptic and millisecond resolution, in real time, as animals engage in various behaviors. For survival, rodents need to identify the smells of objects of interest such as food, mates, and predators, across their recurring appearances in the surround and despite apparent variations in their features. By recording neuronal activity in the input and output layers of the olfactory bulb, as well as from higher olfactory cortical areas, Albeanu and his team aim to understand computations that the bulb performs and how this information is decoded deeper in the brain. They have recently published evidence suggesting that the mouse olfactory bulb is not merely a relay station between the nose and cortex, as many have supposed. Using optogenetic tools and a novel patterned illumination technique, they discovered that the number of information output channels leaving the olfactory bulb for the cortex greatly exceeds the number of inputs received from the nose. They are currently investigating how this diversity of bulb outputs is generated and how the olfactory cortex makes use of such information during behaviors.

The study of decision-making provides a window on the family of brain functions that constitute cognition. Decision intervenes between perception and action and can link one to the other. Although much is known about sensory processing and motor control, much less is known about the circuitry connecting them. Some of the most interesting circuits are those that make it possible to deliberate among different interpretations of sensory information before making a choice about what to do. Anne Churchland's lab investigates the neural machinery underlying decision-making. Her lab uses carefully designed paradigms that encourage experimental subjects, both rodent and human, to deliberate over incoming sensory evidence before making a decision. To connect this behavior to its underlying neural circuitry, they measure electrophysiological responses of cortical neurons in rodents as they perform designated tasks. The lab's current focus is on parietal cortex, which appears to be at the midpoint between sensory processing and motor planning. Churchland and colleagues also use theoretical models of varying complexity to further constrain how observed neural responses might drive behavior. This approach generates insights into sensory processing, motor planning, and complex cognitive function.

Joshua Dubnau and colleagues use the fruit fly to investigate two different questions. First, Dubnau and his team are investigating mechanisms of memory. Because biological mechanisms of memory are highly conserved through evolution, many features of human memory are observed in simpler organisms such as fruit flies. The lab's efforts on memory focus on understanding the gene pathways that are required as well as the neural circuits in the fly brain. They have recently shown

that short-term memory forms in one set of neurons, but that long-term memories form in a different brain area. A second area of research in the Dubnau group is focused on uncovering mechanisms or neurodegeneration that underlie amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Using the fruit fly as an experimental system, together with analyses of genomic data sets from mouse, rat, and human, the Dubnau group has developed a novel hypothesis to explain several different neurodegenerative disorders. Ongoing work in flies and mice will be used to investigate this hypothesis with the goal of developing avenues for therapeutic intervention.

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 and may lead to impairments in memory and depressed mood. In other research, the team has identified the elusive target of deep brain stimulation (DBS), a type of precursor cell that matures into adult hippocampal neurons. 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, and Parkinson's.

Hiro Furukawa's lab is studying neurotransmission at the molecular level. They focus on structure and function of NMDA (*N*-methyl-D-aspartate) receptors, ion channels that mediate excitatory transmission. Dysfunctional NMDA receptors cause neurological disorders and diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, and stroke-related ischemic injuries. NMDA receptors are very large molecules whose three-dimensional atomic structure Furukawa's group has undertaken to solve by dividing them into several domains. The investigators seek to understand the pharmacological specificity of neurotransmitter ligands and allosteric modulators in different subtypes of NMDA receptors at the molecular level. Toward this end, they use cutting-edge techniques in X-ray crystallography to obtain crystal structures of the NMDA receptor domains and validate structure-based functional hypotheses by a combination of biophysical techniques including electrophysiology, fluorescent analysis, isothermal titration calorimetry, and analytical centrifugation. Crystal structures of NMDA receptors serve as a blueprint for creating and improving the design of therapeutic compounds with minimal side effects for treating neurological disorders and diseases. This year, the team discovered and mapped a new regulatory site in a class of NMDA receptors, progress that now opens the way to the development of a potentially new class of drugs to modulate the receptor.

To better understand neuronal circuits, Josh Huang and colleagues have developed novel means of visualizing the structure and connectivity of different cell types at high resolution in living animals and of manipulating the function of specific cell types with remarkable precision. Huang is particularly interested in circuits that use GABA (γ-amino-*n*-butyric acid), the brain's primary inhibitory neurotransmitter. The lab's work has direct implications for neurological and psychiatric illness such as autism and schizophrenia, which involve altered development and function of GABAergic circuits. This past year, Huang's team developed 20 different mouse lines, each of which is engineered to express markers of specific cell types, and demonstrated the exquisite specificity of this technology by imaging GABA cells. The team's work recently has also shed new light on synapse validation, which is at the heart of the process by which neural circuits self-assemble and is directly implicated in neurodevelopmental pathologies. Huang's team looked closely at neurexins, proteins that interact with neuroligins to form the "zipper" that holds synapses together. The team discovered that α and β neurexins respond in different ways to neural activity—the α molecules searching out compatible

connection partners and the β molecules securing preliminary connections that prove to be strong. This adds to past studies in the lab on the underlying framework of molecular guides called glial cells, which nudge nerve fibers to grow in the right direction and make the right contacts. The lab has also made good progress in studying perturbations in the developing GABAergic system in a mouse model of Rett syndrome, an autism spectrum disorder.

Adam Kepecs and colleagues are interested in identifying the neurobiological principles underlying cognition and decision-making. They use a reductionist approach, distilling behavioral questions to quantitative behavioral tasks for rats and mice that enable the monitoring and manipulation of neural circuits supporting behavior. Using state-of-the-art electrophysiological techniques, they first seek to establish the neural correlates of behavior and then use molecular and optogenetic manipulations to systematically dissect the underlying neural circuits. Given the complexity of animal behavior and the dynamics of neural networks that produce it, their studies require quantitative analysis and make regular use of computational models. The team also has begun to incorporate human psychophysics to validate their behavioral observations in rodents by linking them with analogous behaviors in human subjects. Currently, the team's research encompasses study of (1) the roles of uncertainty in decision-making, (2) the division of labor among cell types in prefrontal cortex, (3) how the cholinergic system supports learning and attention, and (4) social decisions that rely on stereotyped circuits. A unifying theme is the use of precisely timed cell-type- and pathway-specific perturbations to effect gain and loss of function for specific behavioral abilities. Through such manipulations of genetically and anatomically defined neuronal elements, the team hopes to identify fundamental principles of neural circuit function that will be useful for developing therapies for diseases such as schizophrenia, Alzheimer's disease, and autism spectrum disorder.

Alexei Koulakov and colleagues are trying to determine the mathematical rules by which the brain assembles itself, with particular focus on the formation of sensory circuits such as those involved in visual perception and olfaction. The visual system of the mouse was chosen for study in part because its components, in neuroanatomical terms, are well understood. What is not known is how projections are generated that lead from the eye through the thalamus and into the visual cortex, how an individual's experience influences the configuration of the network, and which 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, who include Professor Fritz Henn, 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, RNAi-based gene silencing, activation of specific axon terminals using light-gated cation channels, activation or silencing of specific brain regions using transgenes, and assessment of the behavioral consequences of certain manipulations. A project focusing initially on a gene called *ErbB4* seeks to determine the genetic causes of NMDA receptor hypofunction, a pathology believed to contribute to the etiology of schizophrenia. This past year, Li, Henn, and colleagues demonstrated that neurons in a tiny area of the mammalian brain called the lateral habenula (LHb) are hyperactive. They used an analog of deep-brain stimulation to reverse depression-like symptoms in rats.

Partha Mitra seeks to develop an integrative picture of brain function, incorporating theory, informatics, and experimental work. In the ongoing Mouse Brain Architecture Project, Mitra and col-

leagues are well on their way to generating the first-ever brainwide mesoscopic-scale circuit map of the entire mammalian brain. This is a first step toward mapping vertebrate brain architecture across species and in mouse models of human disease, starting with schizophrenia and autism. These maps will be cross-referenced to the Allen Brain Atlas of gene expression, a complementary landmark data set on which the Mitra lab has innovated multiple analyses. These analyses include assessing coexpression patterns and networks of disease-related genes, determining evolutionary ages of genes with respect to their brain region of expression, and correlation with cell-type-specific gene expression. Related collaborative efforts include characterization of different types of GABAergic neurons in the mouse brain, optogenetic studies of the rodent brain, and connectivity mapping in marmoset and zebra finch brains.

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) neural-circuit-based classification of autism and schizophrenia will provide key circuit targets for detailed mechanistic studies and therapeutic development. Osten and colleagues have developed 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 microscopy for whole mouse-brain imaging, called serial two-photon (STP) tomography. 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.

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—and that are likely contributors to disorders such as autism. Shea and colleagues have examined how emotion and arousal enable mice, via their olfactory systems, to store memories of other individuals and of related social signals. The team has exploited the intimate relationship between memory and emotion to effectively create memories in anesthetized mice, allowing them unprecedented access to neurobiological processes that typically only occur during behavior. This year, the lab has been making a detailed analysis of the changes in neural connections that underlie odor memory. The team is particularly focused on an enigmatic cell type that has long been hypothesized to be crucial for memories but has resisted direct study. Team members have developed methods for recording that are giving them the first glimpse of the dynamics of these cells while the animal is learning an odor. The results show unexpected complexity that was independently predicted by a model of odor learning developed in Alexei Koulakov's lab. In parallel, a member of the lab is using imaging techniques to determine how memories are stored among broad neuronal ensembles, as opposed to studying one cell at a time. Also this year, the Shea lab continued a series of studies of a different form of social recognition: auditory recognition of pup vocalizations by their mothers. Through this research, they are beginning their first explicit analysis of a mouse model of Rett syndrome, gathering evidence that these mice exhibit deficits in communication and learning not unlike those in human patients.

What is a memory? When we learn an association, information from two different sensory streams somehow becomes linked together. What is this link in terms of neural activity? For example, after a few bad experiences, we learn that the "green" smell of an unripe banana predicts its starchy taste. How has the neural response to that green smell changed so it becomes linked to that taste? What are the underlying mechanisms—what synapses change strength and what ion channel properties change? These are the questions that drive research in Glenn Turner's lab. His team addresses these questions by monitoring neural activity using a combination of different techniques. Using electrophysiological methods, Turner's team can examine individual neurons with very high resolution,

monitoring synaptic strength and spiking output. The team has also developed functional imaging techniques to monitor the activity of the entire set of cells in the learning and memory center of the fly brain. This comprehensive view of neural activity patterns enables lab members to actually predict the accuracy of memory formation in separate behavioral experiments. By examining the effects of learning-related genes on this process, they can connect their network-level view of memory formation to the underlying molecular mechanisms that govern the basic cellular and synaptic changes that drive learning.

Anthony Zador and colleagues study how brain circuitry gives rise to complex behavior. Work in the lab is focused on two main areas. First, they ask how the cortex processes sound, how that processing is modulated by attention, and how it is disrupted in neuropsychiatric disorders such as autism. Second, they are developing new methods for determining the complete connectivity of the mouse brain at single-neuron resolution. In contrast to previous methods that make use of microscopy, these methods exploit high-throughput DNA sequencing. Because the costs of DNA sequencing are plummeting so rapidly, these methods have the potential to yield the complete wiring diagram of an entire brain for mere thousands of dollars. This past year, Zador published results of research on the influence of expectation on neuronal activity in the rat sensory cortex, suggesting that improvements in auditory perception that result from valid temporal expectation arise from changes in sensory representations as early as the first stages of cortical processing.

Yi Zhong's lab studies the neural basis of learning and memory. The team works with fruit fly models to study genes involved in human cognitive disorders, including neurofibromatosis, Noonan's syndrome (NS), and Alzheimer's disease. Mutations leading to a lack of function of the neurofibromatosis 1 (*NF1*) gene cause noncancerous tumors of the peripheral nervous system as well as learning defects. The lab's analyses of *Drosophila NF1* mutants have revealed how expression of the mutant gene affects a pathway crucial for learning and memory formation. The *NF1* gene and a gene called *corkscrew*, implicated in NS, share a biochemical pathway. Recently, the lab succeeded in linking changes in this pathway due to specific genetic defects in NS with long-term memory deficiencies. In fly models, they discovered the molecular underpinnings of the "spacing effect," the fact that memory is improved when learning sessions are spaced out between rest intervals. Zhong's team has also succeeded in reversing memory deficits in mutant flies—work suggesting that longer resting intervals for NS patients might reverse their memory deficits. This year, the team identified a means of reversing memory loss in fruit flies while suppressing brain plaques similar to those implicated in Alzheimer's disease. Separately, having discovered that memory decay is an active process regulated by the Rac protein, the team has proposed that Rac's role in erasing memory is related to its influence on downstream cytoskeleton remodeling agents.

UNDERSTANDING NEURONAL CIRCUITS IN THE MAMMALIAN OLFACTORY BULB

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The focus of our research group is to understand how neuronal circuits encode and interpret inputs from the environment leading to meaningful behaviors. Toward this end, we are using 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). We want to understand (1) how inputs get processed at different synapses of the underlying neuronal circuits, (2) how these representations change with the state of the system and its circuits (awake vs. anesthetized; naïve vs. learning), and (3) what changes in the activity patterns of well-defined neuronal circuits contribute to specific behaviors. The broad scope of this effort is observing how perceptions arise.

We use the rodent olfactory system as a model and monitor neuronal inputs and outputs in different layers of the circuit. We modulate activity of select neuronal populations in a reversible manner in anesthetized and behaving animals to understand how fundamental sensory encoding problems are solved by the olfactory bulb and piriform cortex networks.

Characterizing the Input-Output Transform of the Olfactory Bulb

In the olfactory bulb (OB), 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 1 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., *Nat Neurosci 12:* 2 [2009]). From each glomerulus, a few dozen mitral cells (principal output neurons of the OB) carry the output farther to the olfactory cortex and several other brain areas. Such "sister" 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). Using optogenetic manipulations and patterned photostimulation (Fig. 2), we found that sister cells carry different information to the cortex: average activity representing shared glomerular input and phase-specific information that refines odor representations and is substantially independent across sister cells (Dhawale et al., *Nat Neurosci 13:* 1404 [2010]).

Activity in the bulb is a rich mix of excitation and inhibition, via both direct inputs and feedback connections. Many different classes of interneurons interact with the sensory afferents and/or the output neurons via both short- and long-range connections. Their connectivity patterns and roles in olfactory processing, however, remain largely unknown. We use a Cre/loxP approach to express reporters (i.e., synaptopHluorin, GCAMP3) and light-gated switches of neuronal activity (Chr2, Halorhodopsin, Arch) in different bulbar neuronal types. We monitor the inputs (glomerular activity patterns) and outputs (mitral cell firing) in response to numerous odorants across a wide range of concentration as we systematically perturb the activity of specific interneuron types. Patterned photostimulation allows us to bypass odor stimulation and gain precise spatiotemporal control over the inputs by directly activating glomerular patterns of choice. We record bulbar outputs via multitetrode recordings and patch-clamp or use optical imaging readouts via multiphoton microscopy in vivo.

We are currently investigating the roles of two classes of interneurons: short axon (SA) cells that broadcast long-range signals in the glomerular layer and granule cells (GCs) that establish reciprocal synapses with mitral/tufted (M/T) cells and receive rich cortical feedback input.

Short Axon Cells Gain Control in the Olfactory Bulb

SA cells, in the glomerular layer, receive inputs from olfactory sensory neurons and/or external tufted (ET) cells and release both GABA and dopamine, synapsing onto juxtaglomerular cells as far as tens of glomeruli away

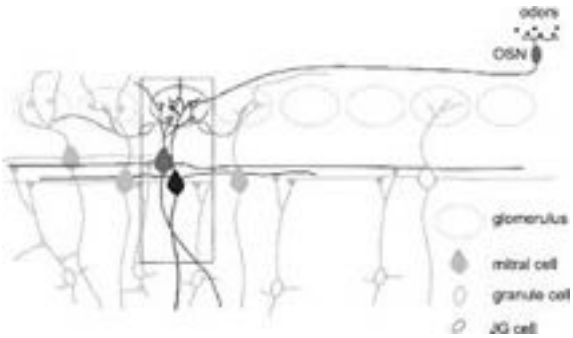

Figure 1. Main olfactory bulb wiring schematics.

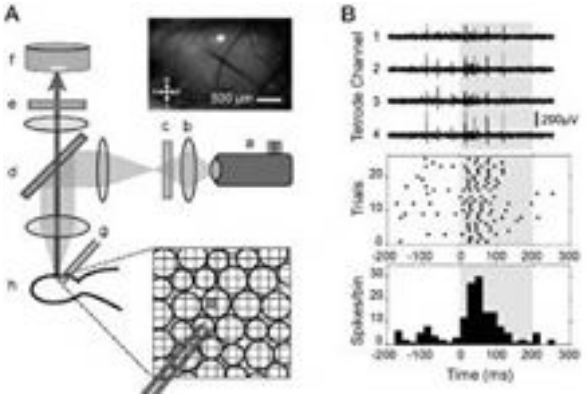

Figure 2. DLP (digital light processing) patterned illumination in ORC (orchiectomized) mice maps the parent glomeruli of mitral cells in vivo. (*A*) (*Left*) A DLP projector coupled to a system of three lenses delivered light patterns to activate individual glomeruli on the dorsal surface of the bulb. A CCD (charge-coupled device) camera was used to visualize the bulb surface and record the position of the light stimulus. (*Top, right*) Dorsal surface of the bulb with a tetrode positioned in the mitral cell layer. One square light spot is projected onto the surface of the bulb. (*Inset*) 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*) 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 showing changes in spiking activity of a single isolated unit across 25 light stimulation trials, each 200 ms long. Dots mark spike occurrence times. (*Bottom*) Peristimulus time histogram (PSTH) with 25-ms time bins, summing spikes over the trials shown in the center panel.

(Kiyokage et al., *J. Neurosci 30:* 1185 [2010]). Computational models (Cleland et al., *Trends Neurosci 33:* 130 [2007]) have suggested that SA cells may be involved in long-range normalization of bulb outputs, but to date, their function in the intact brain has not been investigated. We imaged GCAMP3 responses by wide-field microscopy to odor stimulation across a wide range of concentrations. Odorants induced transient, yet widespread, SA responses, in contrast to focal glomerular patterns observed via intrinsic optical imaging.

To understand the roles played by the SA network on the bulb output dynamics, we recorded extracellularly from M/T cells using tetrodes in anesthetized mice. In conjunction, we selectively activated/inactivated SA cells by shining blue/yellow light either throughout the dorsal bulb surface or in specific spatial patterns. Strikingly, pairing odor presentation at various concentrations with light indicates that SA cells provide both excitatory and inhibitory drive to the M/T cells in a stimulus-specific fashion (Fig. 3). At low odorant concentrations, SA cells provide excitatory input, whereas in response to stronger stimuli, both excitatory and inhibitory drives are present. We are currently performing pharmacological manipulations to dissect the dual roles of SA cells.We propose that the SA network regulates the dynamic range of M/T cell firing by amplifying the weak inputs and down-scaling the stronger ones.

Dissecting the Spatial and Temporal Features of the Glomerular Code in Awake Behaving Animals via Optogenetics Strategies

Odorants activate precise spatiotemporal glomerular activity patterns on the OB surface. These patterns constitute the first representation of odor input into the olfactory system and hence must contain all meaningful attributes of the incoming odor stimulus, such as odor identity, intensity, and temporal dynamics. Different odors activate distinct overlapping and nonoverlapping glomerular maps. In principle, these odor maps can vary in several possible features such as number (how many glomeruli are activated), space (which glomeruli), intensity (absolute and relative levels of activity of individual glomeruli), and timing (onset with respect to each other and the order of their activation). Previous experiments suggest that presenting different odorants (A vs. B) and mixtures (A and B) or different concentrations of the same odorant (A) may all result in modulation of odor maps along many of these (if not all of these) features. It is unknown how the downstream circuitry (mitral/tufted cells) interprets these variations and assigns meaning to them, in order to segregate odor identity from changes in concentration or components within/from a mixture.

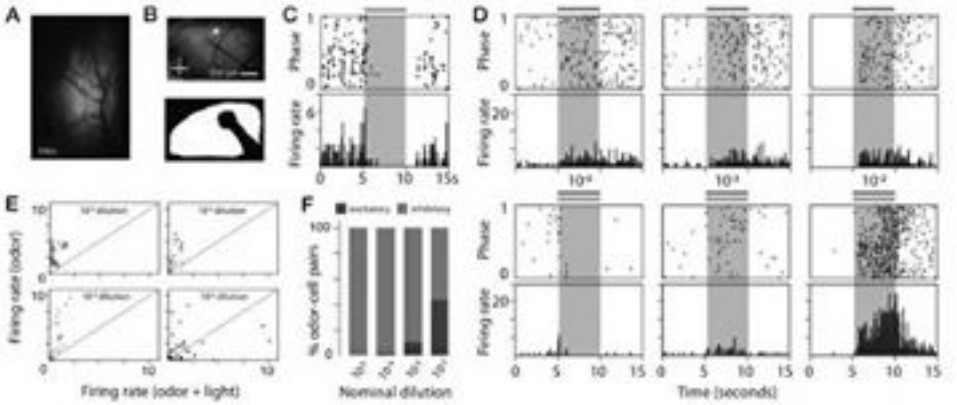

Figure 3. Optogenetic suppression of SA cells reveals both excitatory and inhibitory drive on M/T (mitral/tufted) cell output. (*A*) Homogenous expression of DIO-Halorhodopsin-AAV construct injected in DAT-Cre mice. (*B*) Example of full-field yellow-light mask designed to avoid the recording tetrode. (*C*) Spike raster and PSTH of an M/T cell showing inhibition of spontaneous firing after SA inhibition. Line indicates duration of yellow-light presentation. (*D*) Example of M/T cell response to ethyl valerate alone (*top row*) when paired with full-field yellow light (*bottom row*). Top and bottom lines indicate duration of color and yellow-light presentation, respectively. (*E*) Ratio of firing rate during "odor" and "odor + light" period for 147 M/T cell-odor pairs across different dilutions. (*F*) Percentage of M/T cell-odor pairs at each dilution, which receive excitatory or inhibitory drive from the SA network.

One way to understand how the circuit makes sense of changes in the glomerular activity patterns to extract relevant information is to systematically alter features of odor maps and study the concurrent changes in neuronal outputs and olfactory behavior. Hitherto, however, this has not been possible, primarily because of the inability to activate and modulate individual glomeruli in a controlled manner using odorants. We are using optogenetic tools to bypass odorant stimulation and simulate odor-like glomerular activity patterns, or alter them, by directly activating/inhibiting glomeruli using light in transgenic mice that express Chr2 or Arch in all olfactory system neurons. Furthermore, using patterned illumination, we are activating/inhibiting select subsets of glomeruli with single glomerulus precision in terms of intensity, onset time, or duration. This approach confers an unprecedented ability to make feature-specific perturbations in a glomerular pattern of choice. We aim to understand what features of an odor map are used by a behaving mouse in naturalistic conditions to identify odors and their attributes such as concentrations, temporal fluctuations, or spatial location. Furthermore, we are testing the resolution at which mice can in principle detect variations within specific features of odor maps in a strictly controlled artificial regime. While doing so, we will also monitor neuronal responses at multiple layers in the olfactory system to compare the behavioral and neuronal detection thresholds and to understand the neuronal correlates of olfactory behavior.

Investigating the Roles of Cortical Feedback in Invariant Odor Perception

Invariant perception refers to the identification of a sensory stimulus or an object of interest in a generalized fashion across its variable and recurring presentations. This is a common feature across all sensory modalities and becomes particularly preeminent in olfaction. Natural odor scenes are composed of odor plumes originating from multiple sources and traveling at fluctuating intensities that span several orders of magnitude. Despite this turbulent nature of the stimulus, rodents readily identify odors essential for their survival against varying odor-rich backgrounds and faithfully track a fluctuating odor stream to its source. Several models have attempted to explain invariant perception in different sensory modalities. Some of these models are based on the conventional view of sensory processing that relies solely on feed-forward information flow, whereas others argue that feed-forward mechanisms are insufficient and suggest the involvement of feedback between different neuronal layers. Anatomical studies show massive feedback projections from higher brain areas to the sensory periphery that often outnumber direct sensory inputs. However, the importance of these feedback connections remains largely unknown.

We aim to understand the role of cortico-bulbar feedback (Fig. 4) in the identification of an odor, invariant of fluctuations in its concentration and/or tim-

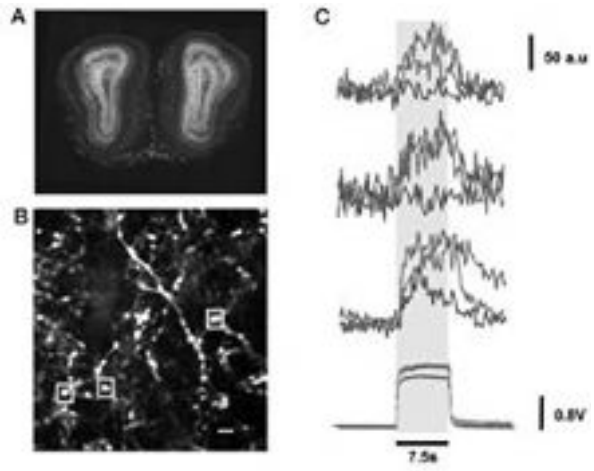

Figure 4. Odor-evoked responses in corticobulbar feedback fibers. (*A*) Corticobulbar feedback fibers labeled with GCAMP3, (*B*) feedback fiber boutons in an optical plane, 230 μm below the surface, (*C*) example of responses to three different odors (ethyl tiglate, 2-heptanone, and p-anis aldehyde) for three synaptic boutons from *B*. Photoionization detector (PID) signals indicate duration of the odor stimulation.

ing and presence of background odors. We are taking advantage of optogenetic tools and patterned illumination techniques recently developed in our group to reversibly manipulate the activity of cortical feedback fibers and their targets in awake head-fixed mice as they learn and perform invariant odor perception tasks. To mimic naturalistic odor conditions, we are making use of custom-designed odor-delivery systems that reproducibly deliver arbitrary time-varying patterns of individual odors and mixtures. To understand the underlying neuronal mechanisms, we are simultaneously monitoring the activity of feedback inputs and their targets via high-speed scanning (~100 Hz) multiphoton microscopy and multitetrode recordings.

Other Collaborative Projects with CSHL Groups

We are collaborating with other fellow CSHL scientists on the following projects: multisensory integration of olfactory and visual information in the mouse brain (A. Churchland); bridging the gap between glomerular odor responses and odor receptor sequences (A. Koulakov and G. Hannon); developing light-sheet-based approaches for fast optical reconstruction of neuronal circuits (P. Osten); and optical monitoring of neuronal activity in genetically and anatomically defined cortical circuits in animal models of cognition (A. Zador).

Florin Albeanu

NEURAL CIRCUITRY FOR PERCEPTUAL DECISIONS

A. Churchland A. Brown D. Raposo
R. Iscowitz J. Sheppard

Making use of sensory information requires more than simply relaying incoming signals from the sensory organs. It requires interpreting information, classifying it, drawing inferences, and ultimately using the context of behavioral goals to make a decision about its meaning. A decision is a commitment to a particular choice or proposition at the expense of competing alternatives. In some situations, decisions involve integration of evidence, i.e., they make use of multiple pieces of information from the environment or from memory. These decisions can provide a framework in which to investigate complex cognitive processes and open a window into higher brain function in general.

Although previous experiments have begun to reveal how neural systems combine evidence to make decisions, they have left a critical gap in our understanding. Specifically, very little is currently known about the neural mechanisms that make it possible to combine information from multiple sensory modalities for decisions. The gap is apparent, although it is clear from behavioral observations that neural systems can combine multisensory information: When parsing speech in a crowded room, for example, the listener makes use of both auditory information (the speaker's vocal sounds) and visual information (the speaker's lip movements). Understanding the neural mechanisms of multisensory integration is critical for two reasons. First, it is essential for a complete understanding of sensory perception because real-world stimuli rarely affect a single sense in isolation. Therefore, understanding how the brain interprets incoming information requires understanding how the brain merges information from different senses. Second, it is likely of clinical importance: Several developmental abnormalities appear to be related to difficulties in integrating sensory information. For example, abnormalities in multisensory processing are a hallmark of subjects with autism spectrum disorder (ASD). Impairments in multisensory processing are also observed in subjects with a collection of sensory abnormalities known together as sensory processing disorder, and they may also be evident in patients with Rett syndrome and dyslexia. Understanding the neural mechanisms of multisensory integration could inform treatment of those conditions.

Our long-term goal is to understand how the brain can make decisions that integrate inputs from our multiple senses, stored memories, and innate impulses. Our current projects, described below, are a first step toward this complete understanding of perceptual decision-making. In the past year, we have made tremendous strides toward achieving this goal. We have successfully developed a behavioral paradigm for studying decision-making in rats and humans (Fig. 1) and have also begun to record electrophysiological responses from the rats while they are engaged in the task. Our first findings with this new paradigm will be published in 2012 in the *Journal of Neuroscience*. We also received an award from the John Merck Fund based on the potential of this research to inform treatment of childhood disorders.

Figure 1. The same decision-making task is used to probe multisensory integration in rats and humans. (*Left*) Schematic drawing of rat in operant conditioning apparatus. Circles are "ports" where the rat pokes its nose to initiate the presentation of sensory stimuli or to report its decision about the stimuli. The white rectangle is the panel of light-emitting diodes that we use to present visual stimuli. An auditory speaker is positioned behind them. (*Right*) Schematic drawing of a human subject seated in front of a computer that we use to present the same auditory and visual stimuli that we use for the rats.

Role of the Parietal Cortex in Multisensory Decision-Making

This work was done in collaboration with David Raposo, Champalimaud Neuroscience Program, Lisbon, Portugal.

The goal of this project is to gain a deeper understanding of the neural mechanisms that enable integration of visual and auditory inputs for decision-making. To this end, we record from neurons in a multisensory area of the rat brain, the posterior parietal cortex. Because we record from neurons during multisensory behavior, we can draw connections between the animal's behavior and the underlying neural mechanism. Although the posterior parietal cortex in rats has previously been explored in the context of navigation, our study is the first study to record from this area on a decision-making task. We use a number of theoretical models to formalize our assumptions about how the neural activity we record might give rise to the behavior.

Probabilistic Representation in the Brain: Insights about Neural Mechanisms that Allow Organisms to Cope with Stimulus Uncertainty

This work was done in collaboration with John Sheppard, Watson School, CSHL.

The goal of this project is to understand how the brain estimates the reliability of sensory stimuli. Previous work in humans has established that they are easily able to estimate the reliability of sensory stimuli. Furthermore, humans use their estimates of reliability to inform the way they combine sensory inputs across modalities. In fact, humans have repeatedly been shown to do this in a statistically optimal way. However, very little is known about the neural mechanisms that make this combination possible. For this project, we have developed a paradigm that presents subjects, both rats and humans, with multisensory stimuli of varying reliability. By measuring subjects' decisions, we can infer the relative weights they assign to one sensory modality or the other. In the rats, we are developing a system to artificially manipulate neural signals in the brain and we plan to investigate how these small manipulations impact subsequent behavior.

Decision-Making in Mice

A. Brown, R. Iscowitz

We are developing a decision-making paradigm using mice instead of rats. The advantage of mice is that there are many tools available that allow us to probe the neural circuitry that underlies decision-making. We have made progress in developing a task that elicits stable, reliable behavior from the mice. A summer student, Ross Iscowitz, began this project and was named an Intel Talent Search Semifinalist for his work on the project. It has been taken over by Amanda Brown, a research associate in the laboratory.

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Churchland AK. 2011. Normalizing relations between the senses. *Nature Neurosci* **14:** 672–673.

In Press

Raposo D, Sheppard J, Schrater P, Churchland A. 2012. Multisensory decision-making in rats and humans. *J Neurosci* (in press).

GENETICS AND NEURAL CIRCUITS UNDERLYING MEMORY AND MEMORY DECLINE IN *DROSOPHILA*

J. Dubnau N. Chatterjee W. Li
M. Cressy L. Prazak
L. Krug H. Qin

The two main areas of research in our lab are (1) to investigate the genetic and neuronal mechanisms of memory using *Drosophila* as a model system and (2) to use the genetics of the fly system to discover the causes of human neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS).

An understanding of memory, indeed of all behavioral phenotypes, will require a multidisciplinary approach to forge conceptual links between the relevant genetic/cell-signaling pathways and 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 normal memory as well as pathological or degenerative cognitive disorders, model systems provide entry points for dissection of cellular mechanisms that are often conserved. Second, systematic manipulation of gene function within relevant anatomical circuits allows a conceptual integration of findings from cellular, neuroanatomical and behavioral levels.

Endogenous Transposable Elements and Neurodegenerative Disorders

N. Chatterjee, L. Krug, W. Li, L. Prazak, J. Dubnau

Transposable elements (TEs) and endogenous retroviruses (ERVs) are mobile genetic elements that provide a massive reservoir of potential genetic instability and toxicity. We have advanced the novel hypothesis that deregulated TE/ERV expression may contribute to TDP-43-mediated neurodegenerative disorders. We have found that TE transcripts are derepressed during the normal aging process. We used mutations in *Drosophila* Argonaute 2 (dAgo2) to create a situation in which TEs are derepressed in the brain. Such mutants exhibit precocious TE elevation in young animals and exacerbated age-dependent derepression. This is accompanied by rapid age-dependent memory impairment and shortened life span. These findings support the hypothesis that protracted loss of TE silencing contributes to age-dependent decline in neuronal physiological function.

In order to investigate the hypothesis that TE derepression contributes to neurodegenerative disorders, we focused on TDP-43, an RNA-binding protein involved in a broad spectrum of neurodegenerative disorders including ALS and frontotemporal lobar degeneration (FTLD). By mining a series of deep-sequencing data sets of protein–RNA interactions and of gene expression profiles, we uncovered broad and extensive binding of TE transcripts to TDP-43. We found that association between TDP-43 and many of its TE targets is reduced in FTLD patients and have discovered that a large fraction of the TEs to which TDP-43 binds become derepressed in mouse TDP-43 disease models. We also have demonstrated that transgenic expression in fly neurons of neurotoxic forms of human TDP-43 causes activation of gypsy, a fly LTR retrotransposon. We propose the hypothesis that TE misregulation contributes to TDP-43-related neurodegenerative disease. Our current efforts are focused on dissection of the underlying mechanisms of transposon control in the brain and the role of unregulated transposon expression in neurodegenerative disorders.

Posttranscriptional Regulatory Network

W. Li, L. Prazak, J. Dubnau

A fundamental property of the brain is that perceptual experiences drive modifications in the number and strength of synaptic connections among neurons. These synapse modifications, which are thought to be neural correlates of memory and cognition, require synthesis of specific proteins at individual synaptic sites in response to neural activity. The mechanisms governing this local synthesis of synaptic proteins are poorly understood. We are using the *Drosophila* model to investigate neuronal translation in two different ways. First, we are developing a novel approach to broadly screen for neuronal translational regulation in terms of both

targets and *trans*-activators. Second, we are focusing on the specific regulatory interactions that we discover to investigate a few translational regulatory pathways in some detail. These efforts are focused on *pumilio*, a translational repressor that we have shown has a role in memory, as well as on RNA-induced silencing complex-mediated regulation.

A global screen for regulatory interactions. We are developing a platform to identify the suite of regulatory mechanisms and translational targets in the brain. The approach involves several steps. The first is a high-throughput method to create in vivo reporters of translation for each gene. The second is a strategy to identify the set of genes whose translation is governed by a particular regulator. As a proof of principle, we will define the targets of five key translational regulators, including Fragile-X protein. Fragile-X is the most common inherited form of mental retardation. Our method, which is conceptually similar to enhancer trapping for transcriptional control, takes advantage of the exceptional genetic manipulability of the *Drosophila* model system. The high degree of conservation of gene regulatory mechanisms and function ensures that much of what we learn will be transferable to humans.

A microRNA–dopamine receptor genetic module in distinct neural circuits for olfactory arousal and olfactory memory. microRNAs (miRNAs) are ~21–23-nucleotide noncoding RNA transcripts that regulate gene expression at the posttranscriptional level. miRNAs regulate gene expression by binding to complementary sequences in the 3′ untranslated regions of target mRNAs. A growing number of studies demonstrate that miRNA biogenesis and function broadly speaking are important for virtually all aspects of cell function, including neuronal function. But there still are relatively few examples where individual miRNA genes have been shown to function acutely in the brain in the context of behavior. We have demonstrated that miR276a expression is required acutely in the *Drosophila melanogaster* brain within two different neural cell types. In both circuits, miR276a appears to target a DA1-type dopamine receptor, but the circuits subserve different aspects of olfactory behavior. This miR276a–dopamine receptor interaction is required in mushroom body (MB) neurons to support long-term olfactory memory and in the ellipsoid body R4 neurons in the central complex to modulate olfactory arousal. This may reveal a conserved functional dissection of memory and arousal in the mushroom body and central complex.

γ Neurons Are the Gateway for DA Input to *Drosophila* MB for Aversive Olfactory Memory Formation

M. Cressy, H. Qin J. Dubnau

MB-dependent olfactory learning in *Drosophila* provides a powerful model to investigate memory mechanisms. MBs integrate olfactory (CS) inputs with neuromodulatory reinforcement (US), which for aversive learning is thought to rely on dopaminergic (DA) signaling to DopR, a D1-like dopamine receptor that is expressed in MBs. A wealth of evidence supports the conclusion that parallel and independent signaling occurs downstream from DopR within two of the three major MB neuron cell types, with each supporting approximately half of memory performance. For instance, expression of the rutabaga adenylyl cyclase (rut) in γ lobe neurons is sufficient to restore learning levels of rut mutants, whereas expression of neurofibromatisis I (NFI) in α/β neurons is sufficient to rescue NFI mutants. Memory consolidation, in contrast, appears to require CREB (cAMP response element binding) and rut signaling in α/β neurons. DopR mutations fully eliminate memory performance, consistent with the hypothesis that DopR receives US inputs for both γ lobe and α/β lobe signaling. We have used spatially restricted expression of the DopR gene to map the neuronal cell types that receive dopaminergic US information underlying this learning task. We have shown that DopR expression in γ neurons is sufficient to fully support short- and long-term memory (Qin et al., in press). Our findings support a model in which DA-mediated CS-US association is formed in γ neurons followed by communication between γ and α/β neurons to drive consolidation. Current efforts are focused on investigation of the role of γ–α/β neuron signaling in memory stabilization/consolidation.

Suppressor Screening by Selective Breeding: The Evolution of Adenylyl-Cyclase-Independent Learning in *Drosophila*

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

Natural populations of animals exhibit remarkably narrow ranges of phenotypic variation relative to the extent of underlying genetic heterogeneity. The phenotypic effects of this hidden genetic variation can be revealed,

however, in response to selective pressure, environmental stress, or the presence of strong deleterious mutations. The clinical severity of Mendelian genetic disorders, for example, can be modulated by variation at additional loci that on their own would have little clinical consequence. To investigate the modulatory impact of such cryptic genetic variation on a Mendelian trait, we used selective breeding over the course of more than 40 generations to evolve nearly normal levels of Pavlovian learning in fruit flies that carry null mutations in the *rutabaga* adenylyl cyclase (M. Cressy et al., in prep.). We constrained the starting genetic variability to a set of 23 loci with known impact in the learning assay, which provided a means to track the underlying genotypic response. We identified eight loci that appear to drive the selection response. By testing the effects of each of the eight loci and all di-allele combinations among them, we demonstrated that at least one locus can partially suppress the rutabaga learning defect on its own. Our findings also support the conclusions that multiple genetic solutions underlie the selected suppression of *rutabaga* and that typical solutions involve interactions among several genes.

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Nabanita Chatterjee

STEM CELLS, SIGNAL TRANSDUCTION, AND BRAIN FUNCTION

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We study stem cells, their contribution to tissue maintenance and regeneration, and signals that control their division and fate. Our main focus is on neural stem cells: We are working to understand how stem cells of the adult brain are regulated during the life span of the organism and how they affect cognitive function, mood, and regeneration of the nervous tissue. Furthermore, we study how other tissue-specific stem cells are regulated, to find commonalities in the mechanisms by which they remain quiescent, self-renew, or differentiate, and to determine whether there is coordination between different types of stem cells in response to physiological stimuli, aging, disease, and therapies. We also study how diffusible signaling molecules affect stem cell differentiation and mediate interactions between stem cells and their environment. Together, these studies may provide an entry point for designing therapeutic interventions to ameliorate the effects of disease or aging, and we are working to translate the results we generate with animal models to human physiology and therapy.

Neural Stem Cells and Brain Disorders

New neurons are continuously generated from multipotent neural stem and progenitor cells in the brain of adult animals and humans. Emerging evidence suggests that adult neurogenesis is important for memory, mood, repair of the nervous system, and pharmacological intervention. Full transformation of a neural stem cell into a functional new neuron is a prolonged process; during this transformation, stem cells and their progeny undergo symmetric and asymmetric divisions, exit the cell cycle, are attacked and eliminated by microglia, express a wide range of markers, change their morphology, and establish connections with other cells. This transition from stem cells to fully integrated neurons, the neuronal differentiation cascade, proceeds through defined steps that can be distinguished through a combination of markers, morphological features, and mitotic activity. Production of neurons from stem cells can be modified by a wide range of intrinsic and extrinsic factors that can positively or negatively affect the outcome.

Our approach to study neural stem cells and their transformation into neurons and astrocytes is based on generating mouse lines in which stem and progenitor cells, as well as their progeny, are highlighted by different fluorescent proteins. These reporter lines enabled us to dissect the differentiation cascade in the adult brain into distinct steps and to assemble a detailed scheme of the cascade (with a focus on the hippocampus, a region of the brain crucial for learning and memory). This scheme provides a conceptual platform upon which to map the targets of various pro- and antineurogenic stimuli and to compare experimental strategies to harness stem cells for tissue maintenance and repair.

We used our scheme of the differentiation cascade to map the cell populations targeted by a number of stimuli, including antidepressant drugs and treatments, physical activity, electroconvulsive shock, deep brain stimulation (DBS), radiation, chemotherapeutic agents, cortical trauma, ablation of dopaminergic neurons, and aging. In particular, we analyzed the effect of DBS of the anterior thalamic nucleus (ATN) and the cingulate gyrus (CG) on hippocampal neurogenesis. DBS is being increasingly used to treat neurological and psychiatric disorders; recently, it has been successfully applied to patients with treatment-resistant depression or in a minimally conscious state. In addition to its clinical importance, DBS presents a powerful approach to target specific neural circuits and to determine the functional relationship between the components of these circuits. We examined the effect of high-frequency stimulation of crucial components of the limbic circuitry, the ATN and the CG, on the generation of new neurons in the dentate gyrus (DG), another component of the same circuitry. Using our reporter mouse lines designed to quantify changes in selected classes of neural progenitors, we found that high-frequency stimulation of the ATN increases symmetric divisions of amplifying progenitors, a defined class of neural progenitors in the DG; this effect is later manifested as an increased number of new neurons. The same class of

neural progenitors is also affected by the antidepressant fluoxetine (Prozac) and physical activity (running). This confirms that neurogenic stimuli of different natures can converge on the same neurogenic target in the DG. Our results also suggest that hippocampal neurogenesis may be used as a sensitive indicator of the limbic circuitry activation induced by DBS.

Overall, our results demonstrate that various pro- and antineurogenic stimuli have defined neurogenic targets that may or may not overlap. Importantly, we found that most of the treatments used for the therapy of depression, while increasing adult hippocampal neurogenesis, do not affect the pool of stem cells, acting instead on the downstream steps of the cascade (e.g., increasing the number of divisions of the transiently amplifying progenitor cells). In contrast, trauma and loss of dopaminergic neurons lead to a transient activation of stem cells; although this effect may reflect a compensatory response of the damaged brain, it may also lead to a premature exhaustion of the stem cell pool.

Our results also helped to elucidate the most basic mechanism of stem cell control in the adult and aging brain. It has been known that there is a profound age-related decline in the number of new neurons; given the significance of new neurons for cognitive function, it has been speculated that age-related decrease in hippocampal neurogenesis contributes to age-related cognitive impairment. However, it was not known whether the reason for the decrease is the diminished efficiency of stem cells or a decrease in their number. We used reporter lines, lineage tracing, new techniques for cell division kinetics analysis, and computational modeling to show that age-related decrease in hippocampal neurogenesis is driven by the disappearance of neural stem cells via their conversion into mature astrocytes. We also found that the astrocytic differentiation (and the loss of stem cell properties) of neural stem cells is coupled to a rapid series of their asymmetric divisions. This finding stands in great contrast to the conventional model of stem cell maintenance and division, which assumes multiple cycles of activation and quiescence of stem cells. We proposed a "disposable stem cell" model which posits that an adult hippocampal stem cell is quiescent throughout the entire postnatal life, but, when activated, undergoes several rapid asymmetric divisions (with the daughter cells eventually producing neurons) and then leaves the pool of stem cells by converting into an astrocyte. This model describes the life cycle of an adult neural stem cell and reconciles numerous observations on the age-related decrease in production of new neurons, the age-related increase in astrocyte number, and the disappearance of hippocampal neural stem cells. Together, these continuous changes underlie age-dependent decrease in production of new neurons and may contribute to age-related cognitive impairment. Interestingly, we found that although the number of hippocampal neural stem cells gradually declines, their efficiency (their retention rate and production of progeny per cell) gradually increases, possibly reflecting a strategy of coping with the consequences of aging.

This model has important implications for understanding the mechanisms of stem cell maintenance and division in the adult brain; furthermore, it may also shed light on the modes of stem cell maintenance in other tissues. In particular, the model indicates that excessive activation of stem cells (rather than their progeny) during multiple brain traumas or Parkinson's disease may lead to a premature exhaustion of the stem cell pool and may eventually impact cognitive abilities.

Stem Cells in Nonneural Tissues

Tissues and organs undergo continuous remodeling in response to stress, damage, or physiological demand. Adult tissue-specific stem cells are located in specialized niches that restrict their division and support their undifferentiated status. We found that the same mouse reporter lines which we used to identify stem cells of the nervous system can also identify stem and progenitor cells in a range of other tissues and organs. This list includes stem cells of such dissimilar structures as brain and spinal cord, hair follicles, liver, pancreas, skeletal muscle, testis, retina, anterior pituitary, skin, and bone marrow. This provides a means of visualizing stem and progenitor cells, tracing their lineage, isolating them, and studying molecular mechanisms that control their quiescence, division, self-renewal, and differentiation. Using this reporter-based approach, we performed collaborative experiments with Dr. Paul Frenette's (Albert Einstein College of Medicine) and Dr. Sean Morrison's (University of Texas Southwestern) groups to identify mesenchymal stem cells in the bone marrow and to demonstrate that they contribute to the maintenance of the hematopoietic stem cells niche. These two types of stem cells are found in close association; furthermore, depletion of mesenchymal stem cells results in a reduced ability of hematopoietic stem cells to home in on the bone marrow. This is the first example of a partnership between two distinct somatic stem cell types. Such combinations of heterotypic stem cells may exist in other tissues as well and may help coordinate the re-

modeling of a tissue or an organ; we are currently working to identify these combinations.

Most recently, to visualize signaling events that control stem cell maintenance and division, we generated transgenic mouse lines that express genetically encoded sensors of redox signaling. When combined with the reporter lines, the sensor/reporter combinations enable visualization of specific signaling events in stem and progenitor cells and their progeny or in the vicinity of these cells. We work to expand the panel of sensors and reporters to visualize multiple signaling events controlling stem cells.

Nitric Oxide, Development, and Differentiation

Much of our research interest is focused on the biological role of a versatile signaling molecule: nitric oxide (NO). During the last few years, we discovered that NO has an essential role in development and in stem cell regulation. We found that in diverse settings, ranging from *Drosophila* larvae to mouse brain, NO acts as a regulator of the balance between cell quiescence, division, and differentiation, eventually controlling tissue formation and remodeling. Furthermore, we found that NO also regulates vertebrate morphogenesis by linking together suppression of cell division and facilitation of cell movements. We also demonstrated that NO controls these two processes through two separate signaling pathways, helping to ensure that the crucial processes of cell proliferation and morphogenetic movements are tightly coordinated during early development.

We continue to discover the diversity of the biological functions of NO. In our latest studies, we found that in such diverse settings as the skin of the frog embryo and mammalian airways, production of NO in multiciliated cells is crucial for the establishment of the cell- and tissue-level polarity and cilia growth, and, eventually, for the generation of the directed flow of fluid across the tissue. We also found that NO acts upon two distinct signaling pathways to control the polarity of cilia distribution and the cilia length, and we identified the critical targets of NO signaling in both pathways. The remarkable conservation of the NO role in the function of cilia in different developmental contexts suggests that some of the human ciliopathies and related disorders may be associated with the inadequate activity of NO synthase and may benefit from NO-based therapies.

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MOLECULAR ANALYSIS OF NEURONAL RECEPTORS AND ION CHANNELS

H. Furukawa A. Jespersen E. Karakas N. Simorowski P. Singh N. Tajima

The research program in my group aims at understanding the molecular basis for the functions of cell surface receptors or membrane proteins that initiate cellular signal transductions involved in neurotransmission in the mammalian brain. We hope that this work will support the design of compounds with therapeutic potential 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, two classes of membrane proteins that regulate the processes of neurotransmission and the dysfunction of which is implicated in neurodegenerative diseases. To achieve our goals, we use X-ray crystallography to determine the three-dimensional atomic structures of target proteins and test structure-based mechanistic hypotheses by site-directed mutagenesis in combination with biochemical and biophysical techniques. Our main focus in 2011 was to obtain high-resolution structures of the extracellular domains of a class of ligand-gated ion channels, *N*-methyl-D-aspartate receptor (NMDAR), in complex with lead compounds with the potential to protect neurons. Based on the structural information obtained, we have validated our functional hypotheses using a combination of techniques including electrophysiology, isothermal titration calorimetry, and analytical ultracentrifugation.

Structural Dissection of NMDAR Pharmacology

NMDARs belong to the family of ionotropic glutamate receptors (iGluRs) that mediate the majority of excitatory synaptic transmissions in the mammalian brain. They are voltage-sensitive calcium ion channels composed of two copies each of the GluN1 and GluN2 subunits, which bind glycine and glutamate, respectively. Calcium signals facilitated by NMDARs have a pivotal role in cellular signal transduction resulting in the neuroplasticity essential for learning and memory formation. The four distinct GluN2 subunits (A–D) control the pharmacological properties of NMDAR ion channels and show different spatial and temporal expression patterns; thus, there has been a great interest in creating subtype-specific compounds that can target specific neuronal circuits.

The NMDAR subunits are modular and are composed of different protein domains, including the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TM), and carboxy-terminal domain (CTD) (Fig. 1). Our current studies are focused on ATD and LBD; these domains mediate allosteric regulation and activation in the NMDAR ion channels upon binding to polyamine-based compounds and neurotransmitters, respectively. Among all of the ATD-targeting compounds, those that specifically recognize GluN1/

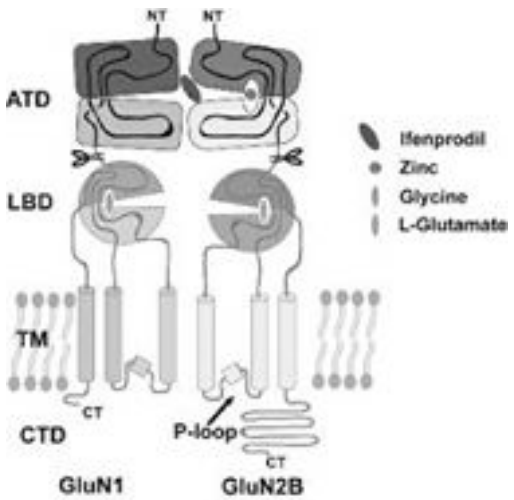

Figure 1. Domain organization of NMDAR subunits, modular proteins composed of distinct domains including the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TM), and carboxy-terminal domain (CTD). Shown are schematic presentations of the glycine-binding GluN1 subunit and the L-glutamate-binding GluN2B subunit. Allosteric modulators such as phenylethanolamine and zinc bind ATD.

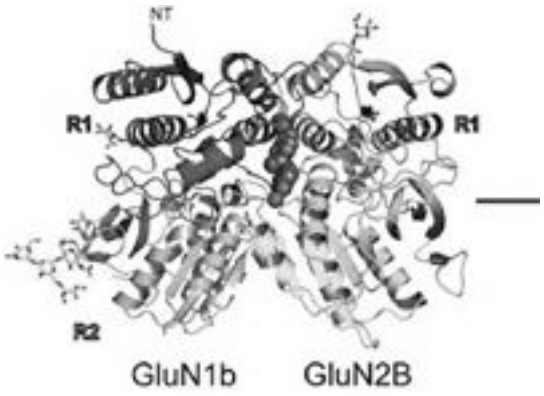

Figure 2. Crystal structure of the recently solved GluN1/GluN2B NMDAR ATD structure. GluN1 (*left*) and GluN2B (*right*) ATDs have bilobed structures composed of R1 and R2 domains and form heterodimers. Ifenprodil (spheres) bind to the subunit interface between GluN1 and GluN2B. Sticks indicate glycosylation sites.

GluN2B NMDARs (e.g., ifenprodil) have drawn substantial interest because of their therapeutic potential in stroke-related brain injuries, depression, and Alzheimer's disease. Until 2011, the molecular mechanism for ATD-mediated regulation was not well understood, and structural information for the NMDAR ATD had been limited to the GluN2B ATD monomer in complex with zinc (recently published by my group). Thus, the precise mapping of the location of the ifenprodil-binding site within GluN1/GluN2B NMDARs has been met with much enthusiasm. This strategy may provide crucial information for improving the design of ifenprodil to minimize side effects.

We have also completed X-ray crystallographic studies on the GluN1/GluN2B ATD in complex with ifenprodil; thus, we are now able to locate the ifenprodil-binding site for the first time (Fig. 2). The ifenprodil-binding site identified through this study is distinct from the previously predicted site that involved only the GluN2B subunit. Our study shows the first "correct" model of the ifenprodil-binding site.

To further understand the ifenprodil-binding site, we wanted to identify the structure of the GluN1/GluN2B in complex with Ro-25-6981, a significantly (~10-fold) more potent ifenprodil analog that has become commercially available in recent years. Structural comparison revealed the molecular explanation for the higher affinity of Ro-25-6981 compared to ifenprodil and provided a possible strategy for improving the design of the ifenprodil-like compounds for higher specificity and potency. The binding site of Ro-25-6981 is located at the subunit interface of GluN1/GluN2B ATD and is mediated by amino acid residues from both GluN1 and GluN2B subunits in a manner similar to that of ifenprodil (Fig. 3).

We have conducted structure-based site-directed mutagenesis to validate the physiological relevance of the binding site by measuring currents elicited by the mu-

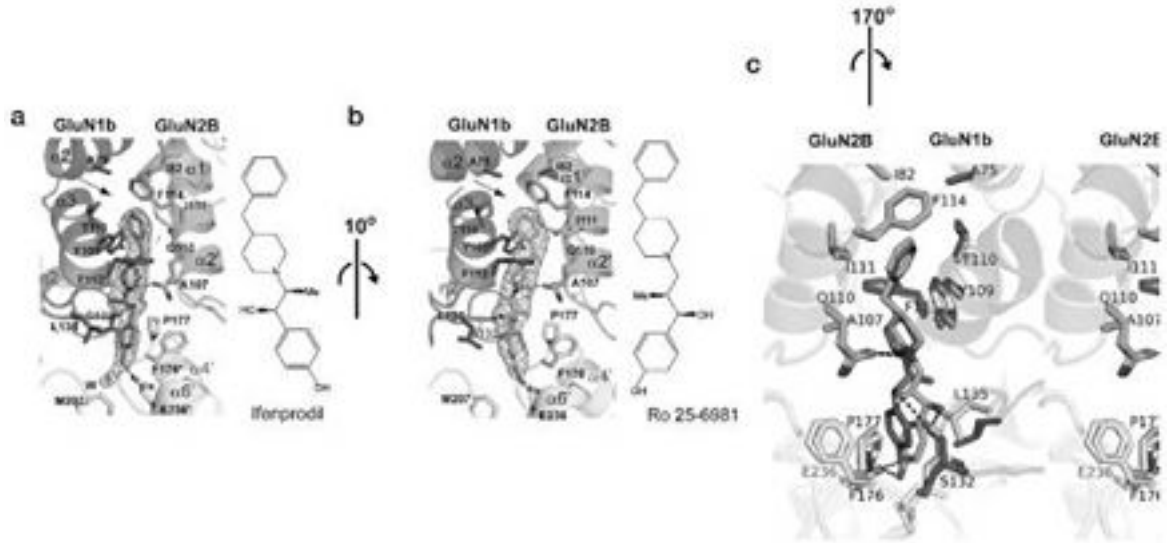

Figure 3. Phenylethanolamine-binding site. Binding modes of ifenprodil (*a*) and Ro-25-6981 (*b*). Shown in mesh is the electron density (*Fo-Fc* omit electron map) contoured at 3s. (*c*) Comparison of binding sites for ifenprodil and Ro-25-6981. van der Waal contacts are stronger for binding of Ro-25-6981 than ifenprodil and probably account for the higher potency of Ro-25-6981.

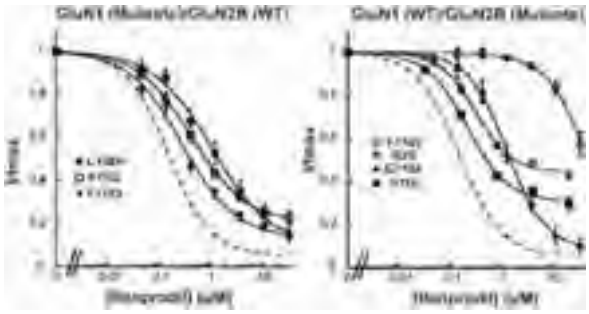

Figure 4. Mutagenesis at phenylethanolamine-binding site. Single point mutations are incorporated into GluN1 or GluN2B at the phenylethanolamine-binding site. All of the mutations cause a change in either potency or efficacy. This indicates that the phenylethanolamine-binding site observed in the crystal structure is physiologically relevant.

tants. Seven residues around the binding pocket were mutated to partially disrupt the binding pocket. All such mutants showed a shift in either potency or efficacy of ifenprodil inhibition, indicating that the ifenprodil- and the Ro-25-6981-binding sites are physiologically relevant (Fig. 4).

To understand the molecular mechanism of allosteric inhibition mediated by ifenprodil, we have conducted biochemical/electrophysiological experiments on the full-length GluN1/GluN2B NMDARs. Toward this end, we have designed cysteine mutants that disulfide-cross-link GluN1 and GluN2 subunits at the interfaces, thereby "trapping" the conformation of GluN1 and GluN2B ATDs (Fig. 5a). The current recording on the mutant NMDARs by electrophysiology showed that the disulfide cross-link that restricts the motion of a portion of GluN2B called R2 results in locking the NMDAR ion channels to the "inhibited" state. These experiments led to a model that the GluN2B ATD is at a conformational equilibrium between "open-cleft" and "closed-cleft" conformations, where the former represents the active state of the NMDAR channel and the latter represents the inactive state (Fig. 5).

Structure and Function of γ-Secretase

Another major project that we are deeply involved in is the structural and functional study of a molecular complex called γ-secretase, which is implicated in initiation of Notch signaling critical for cell differentiation and stem cell maintenance as well as production of β-amyloid. Formation of neurotoxic β-amyloid oligomers and deposition of β-amyloid fibril are hallmarks of Alzheimer's disease. Furthermore, overactivation of Notch signaling is frequently observed in various types of cancers. Thus, fine regulation of γ-secretase may be an effective strategy in the treatment of those major diseases.

γ-Secretase is a multimeric protein complex of four transmembrane proteins including presenilin, nicastrin, APH-1, and PEN-2 (Fig. 6). Together, the protein complex mediates an unusual molecular event called regulated intramembrane proteolysis, in which the transmembrane substrate is proteolysed within the biological membrane. This multimembrane protein complex cleaves a number of single transmembrane substrates, including the Notch ligand and amyloid precursor protein.

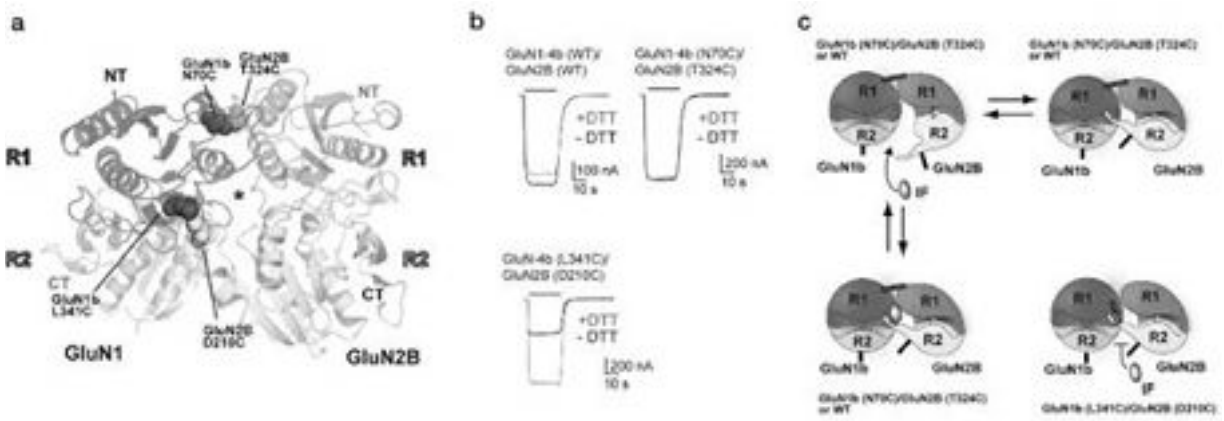

Figure 5. Engineering intersubunit disulfide bonds trap the NMDAR ion channel in inhibited state. (*a*) Location of intersubunit disulfide bonds. (*b*) Macroscopic current recording in the presence (gray) and absence (black) of DTT. (*c*) Possible model of ifenprodil binding and the movement of ATDs coupled to allosteric inhibition.

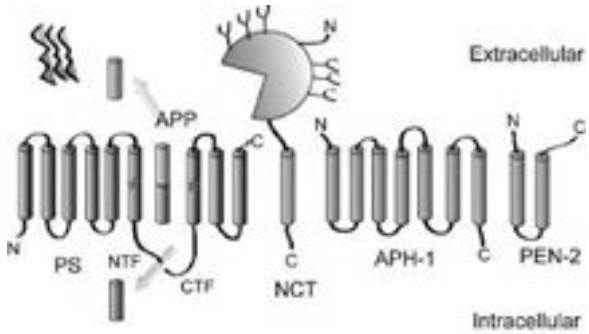

Figure 6. Components of γ-secretase, a protease complex composed of four integral membrane proteins: presenilin (PS), nicastrin (NCT), APH-1, and PEN-2. The wild-type PS contains active site aspartic residues (asterisks) within the transmembrane (TM) domains (TM6 and TM7) and becomes active only when it forms a complex with three other components. A single transmembrane substrate such as amyloid precursor protein (APP) is proteolytically processed within the membrane. The extracellular and cytosolic domains form neurotoxic β-amyloid oligomers or act as a transcriptional regulator, respectively. NCT and APH-1 are considered to be important for substrate recognition, whereas PEN-2 has a critical role in proteolysing PS at the cytosolic loop between TM6 and TM7, thereby converting PS into the active form.

The mechanistic understanding of intramembrane proteolytic activities in γ-secretase is limited due to a lack of information about its atomic structure. Our research program is aimed at obtaining an atomic map of γ-secretase to gain insight into intramembrane proteolysis, substrate specificity, inhibition, and functional modulation.

Our group is also interested in determining the atomic structure of γ-secretase to gain insight at the molecular level. Currently, we are working toward understanding the mechanism of substrate recognition mediated by one of the γ-secretase components, nicastrin. Structural and functional studies on γ-secretase have the potential of leading to development of compounds that can be used to lower the level of neurotoxic amyloid oligomers in Alzheimer's disease as well as to alleviate overactive Notch signaling in cancer.

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Erkan Karakas

ASSEMBLY AND FUNCTION OF GABA INHIBITORY CIRCUITS IN THE NEOCORTEX

Z.J. Huang Y. Fu, K. Krishnan, J. Lu, J. Tucciarone, M. He, M. Lazarus, A. Paul, P. Wu, S. Kelly, Y. Lin, H. Taniguchi

We combine genetic, imaging, and physiology approaches to study the organization, function, and development of neural circuits in cerebral cortex, focusing on GABAergic inhibitory interneurons.

Genetic Dissection of GABAergic Circuits in Neocortex

In the mammalian neocortex, the delicate balance and dynamic assembly of the functional architecture of neural circuits are achieved through a rich repertoire of inhibitory control mechanisms. A key obstacle to understanding cortical circuits is the diversity of GABAergic interneurons, which poses general questions in neural circuit analysis: How are diverse cell types generated and assembled into stereotyped local circuits? How do they differentially contribute to circuit operations that underlie cortical functions ranging from perception to cognition? Using genetic engineering and the Cre/LoxP strategy in mice, we have generated and characterized more than 20 Cre and Flp knockin driver lines that reliably targeted major classes and lineages of GABAergic neurons. More distinct populations are captured by intersection of Cre and Flp drivers and by engaging lineage and birth-timing mechanisms. Genetic targeting allows reliable identification, monitoring, and manipulation of GABAergic neurons, thereby enabling systematic and comprehensive analysis from cell-fate specification to connectivity and function in networks and behavior.

The Origin and Organization of Chandelier Cells in Mouse Neocortex

The precise control of action potential initiation at the axon initial segment (AIS) determines whether and when a neuron transmits its information and allows highly coordinated output in a neural ensemble. In mammalian cerebral cortex, AISs of pyramidal neurons are regulated by GABAergic synapses from a highly distinct class of interneuron, the axon-axonic cells or chandelier cells (ChCs), which increase their abundance in primates and human. A single ChC innervates hundreds of pyramidal neurons and might exert decisive influence in their firing, but the developmental origin and cortical organization of ChCs remain unknown. Previous studies in rodents suggested that most if not all cortical GABAergic neurons derive from the medial and caudal ganglionic eminence (MGE and CGE) of the basal ganglia and the preoptic area, mainly during midgestation. We discovered that ChCs in the mouse predominantly arise from progenitors expressing the homeodomain protein Nkx2.1 located in the ventral tip of the late embryonic subventricular zone (SVZ), after the disappearance of MGE. Postmitotic ChCs migrate to their cortical destination with stereotyped route and schedule, but they do not follow the inside-out (developmental) sequence of laminar deployment observed by pyramidal neurons and MGE-derived interneurons. Mature ChCs show striking laminar enrichment in layers 2 (L2), 5, and 6 and are more abundant in mediofrontal area compared to sensory cortices. L2 ChCs selectively innervate the distal portion of pyramidal cell AISs, the exact site of spike initiation. Nearby L2 ChCs are frequently electrically coupled and can further activate one another through disynaptic excitation, suggesting a coordinated functional network. Our results demonstrate protracted neurogenesis and a novel progenitor source in the Nkx2.1 lineage that gives rise to a functionally powerful and evolutionarily expanding class of cortical interneuron. Genetic targeting of ChCs provides an entry point to studying cortical circuit assembly, organization, and dysfunction.

GABA Signaling Promotes Synapse Elimination and Axon Pruning in Developing Inhibitory Neurons

Accumulating evidence indicates that GABA acts beyond inhibitory synaptic transmission and regulates the development of inhibitory synapses in the vertebrate brain, but the underlying cellular mechanism is not well

understood. We have combined live imaging of cortical GABAergic axons across timescales from minutes to days with single-cell genetic manipulation of GABA release to examine its role in distinct steps of inhibitory synapse formation in the mouse neocortex. We have previously shown, by genetic knockdown of GABA synthesis in developing interneurons, that GABA signaling promotes the maturation of inhibitory synapses and axons. We found that a *complete blockade* of GABA release in basket interneurons resulted in an opposite effect—a cell-autonomous *increase* in axon and bouton density with apparently normal synapse structures. These results not only demonstrate that GABA is unnecessary for synapse formation per se, but they also uncover a novel facet of GABA in regulating synapse elimination and axon pruning. Live imaging revealed that developing GABAergic axons form a large number of transient boutons, but only a subset was stabilized. Release blockade led to significantly increased bouton stability and filopodia density, increased axon branch extension, and decreased branch retraction. Our results suggest that a major component of GABA function in synapse development is transmission-mediated elimination of subsets of nascent contacts. Therefore, GABA may regulate activity-dependent inhibitory synapse formation by coordinately eliminating certain nascent contacts while promoting the maturation of other nascent synapses.

Presynaptic $GABA_B$ Receptor Regulates Activity-Dependent Maturation of Inhibitory Synapses

Accumulating evidence indicates that GABA regulates activity-dependent development of inhibitory synapses in the vertebrate brain, but the underlying mechanisms remain unclear. Here, we combined live imaging of cortical GABAergic axons with single-cell genetic manipulation to dissect the role of presynaptic $GABA_B$ receptors ($GABA_B$Rs) in inhibitory synapse formation. Developing GABAergic axons form a significant number of transient boutons, but only a subset was stabilized. Synaptic vesicles in these nascent boutons are often highly mobile in the course of tens of minutes. Activation of presynaptic $GABA_B$Rs stabilized mobile vesicles in nascent boutons through the local enhancement of actin polymerization. Inactivation of $GABA_B$Rs in developing basket interneurons resulted in an aberrant pattern of bouton size distribution, reduced bouton density, and reduced axon branching, as well as reduced frequency of miniature inhibitory currents in postsynaptic pyramidal neurons. These results suggest that $GABA_B$Rs along developing inhibitory axons act as a local sensor of GABA release and promote presynaptic maturation through increased recruitment of mobile vesicle pools. Such release-dependent validation and maturation of nascent terminals is well suited to sculpt the pattern of synapse formation and distribution along axon branches.

Impact of GABAergic Deficiency in Synaptic Transmission and Behavior

A highly reproducible observation in schizophrenia (SZ) is the reduction of mRNA for GAD67, the principle enzyme for GABA synthesis in cortex, in certain types of cortical interneurons, particularly those expressing parvalbumin (PV). GAD67 expression is regulated by neural activity, and its reduction in SZ is most likely a downstream impact of reduced engagement of the PV cell network. Because PV cells have an important role in the generation and maintenance of gamma wave activity, dysfunction in these cells is thought to contribute to cognitive deficits in SZ, such as working memory impairment. To model the cell-type-specific changes observed in SZ, we used a cre/lox system in mice to conditionally knock down *GAD67* in PV cells. Virus-vector-mediated green fluorescent protein (GFP) expression was used for paired recordings from PV to pyramidal cells, and behavioral tests, including Y-maze spontaneous alternation, to assess working memory function. In young mice, reduction of GAD67 in PV cells led to weakened synaptic inhibition. Removal of one allele of *Gad1* (heterozygote) led to a 33% reduction of GAD67 in PV cells and further caused a supralinear deficit in synaptic transmission. Young mutant mice also demonstrated a behavioral phenotype featuring reduced spontaneous alternation in the Y maze. Additionally, pyramidal cells demonstrated hyperactive spiking properties in response to current injection. In adult mice, however, both the synaptic and behavioral deficits had resolved, leading the mutant mice to be indistinguishable from controls in all measured parameters. We therefore have demonstrated that GABA deficiency does in fact lead to weakened synaptic transmission, with a concurrent behavioral phenotype. However, weakened inhibition in juvenile mice appears to not produce any irreversible changes, as homeostatic mechanisms were able to fully eliminate the impact of this artificially induced GAD67 deficit. Our study suggests that PV-cell function, or possibly GAD67 expression level itself, presents a possible treatment target for cognitive dysfunction.

Distinct DNA Methylation Signatures in Glutamatergic and GABAergic Neurons of the Cerebral Cortex

A critical role for DNA methylation has been implicated in cell differentiation and development and recently in behavioral plasticity in postmitotic neurons. A key obstacle in genomic and epigenomic analysis in the brain is cellular heterogeneity—genomes of distinct yet highly intermingled cell types are largely inaccessible to sequencing technology. To address this issue, we have generated a set of cell-type-specific Cre knockin lines that activate a nuclear-GFP reporter. Using FACS (fluorescence-activated cell sorting) of dissociated cortices and whole-genome bisulfite sequencing, we have analyzed the DNA methylomes of two cardinal neuron types, the glutamatergic and GABAergic neurons. We achieved 10 times whole-genome coverage per cell type, allowing us to interrogate ~90% of the CpG sites in the mouse genome. A hidden Markov model allowed us to identify hypomethylated regions (HMRs) in the two methylomes. Although similar numbers of HMRs were identified in both cell types in the transcription start sites, glutamatergic cells had significantly higher HMRs in the intragenic and intergenic regions. These HMRs encompass previously defined CpG islands, but we also find significantly more previously undetected HMRs. To determine if these are neuron-specific, we are comparing these methylomes with those of microglia cells, which are derived from a distinct lineage. As intragenic hypomethylation likely has a role in regulating gene expression, we are currently analyzing the differential gene expression patterns of these cell types using strand-specific RNA-Seq and RT-PCR (reverse transcriptase–polymerase chain reaction) on select candidate genes. Interestingly, genes in several prominent neuronal pathways such as axon guidance and signaling (WNT, MAPK) are differentially methylated between the two cell types. Our approach establishes a cell-type-based experimental paradigm for epigenomic analysis in complex tissues and sets the stage for analysis of neuronal subtypes during both brain development and behavioral plasticity.

Cell-Type-Based Analysis of microRNA Profiles in the Mouse Brain

microRNAs (miRNAs) are implicated in brain development and function, but the underlying mechanisms have been difficult to study in part due to the cellular heterogeneity in neural circuits. To systematically analyze miRNA expression in neurons, we have established an miRNA tagging and affinity purification (miRAP) method that is targeted to cell types through the Cre-loxP binary system in mice. Our studies of the neocortex and cerebellum reveal the expression of a large fraction of known miRNAs with distinct profiles in glutamatergic and GABAergic neurons and subtypes of GABAergic neurons. We further detected putative novel miRNAs, tissue- or cell-type-specific strand selection of miRNAs, and miRNA editing. Our method thus will facilitate a systematic analysis of miRNA expression and regulation in specific neuron types in the context of neuronal development, physiology, plasticity, pathology, and disease models and is generally applicable to other cell types and tissues.

A Novel Function of MeCP2 in mRNA Translation in the Brain

MECP2 was identified as a nuclear methyl-CpG DNA-binding protein and is best known as a regulator of transcription and chromatin. Mutations in MECP2 are linked to autism spectrum disorders, especially the Rett syndrome (RTT), but the pathogenic mechanisms remain elusive. Here, we report on a translation regulatory function of MECP2. MECP2 is detected in discrete puncta of neuronal cytoplasm, in brain polyribosomes, and interacts with numerous messenger ribonucleoproteins with translation regulatory functions. MECP2 associates with hundreds of mRNAs, which, in its absence, show altered translation and encoded protein levels. Importantly, actively translating mRNAs, but not the steady-state transcript levels, show pronounced changes in *MeCP2*-null brain. The MECP2-regulated mRNAs are involved in synaptic transmission, cell signaling, and ion homeostasis and likely contribute to a range of neural and physiological processes deficient in RTT. Our results reveal a novel biological function of MeCP2 and implicate translation regulation in RTT pathogenesis.

GABAergic Interneurons and Neurodevelopmental Disorders

We are studying alterations of GABAergic inhibitory circuits in mouse models of RTT, one of the autism spectrum disorders, caused by mutations in the X-linked gene *MeCP2*. Our general hypothesis is that

MeCP2 mutations perturb the postnatal maturation of connectivity, function, and plasticity in subsets of inhibitory interneurons in distributed brain areas, leading to altered development and maladaptive plasticity of neural circuits and characteristic behavioral deficits.

Neuronal circuits in the brain are shaped by experience during "critical periods" in early postnatal life. In the cortex, this activity-dependent development is coincident with the functional maturation of parvalbumin (PV^+) GABAergic interneurons, although the underlying mechanisms are unclear. We have obtained substantial evidence that *MeCP2* regulates the timing of a critical period of plasticity in the visual cortex, likely through the maturation of the GABAergic neurons. We found that loss of methyl-CpG-binding protein (MECP2) results in accelerated maturation of PV^+ fast-spiking cortical interneurons, likely resulting in a shift in critical period onset using molecular markers and physiological parameters. Furthermore, loss of *MeCP2* in PV^+ interneurons resulted in increased expression of the rate-limiting enzyme GAD67, which synthesizes GABA, the major inhibitory neurotransmitter in the brain. Our current results indicate a requirement for *MeCP2* in regulating the development of activity-dependent maturation of PV^+ GABAergic interneurons via GAD67, which in turn innervate the perisomatic region of pyramidal neurons and control their output. As *MeCP2*-null mice serve as a model for RTT, our findings provide insight into the inappropriate synaptic plasticity underlying the pathogenesis of this disease. Altered timing of critical period might lead to deficits in experience-dependent associations in other modes of perception including multisensory integration, social interaction, and language skills, as seen in RTT and other neurodevelopmental disorders.

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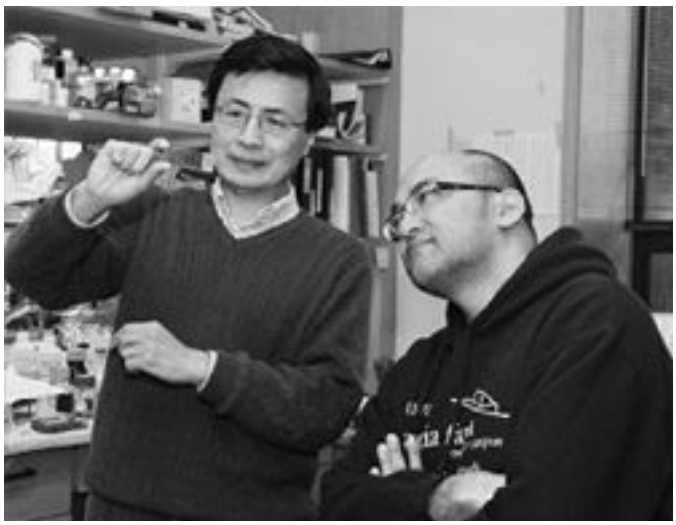

Z. Josh Huang and Hiroki Taniguchi

NEUROBIOLOGY OF COGNITION AND DECISION-MAKING

A. Kepecs E. Demir J. Hirokawa S. Ranade
M. Faleiro D. Kvitsiani J. Sanders
B. Hangya H.J. Pi A. Vaughan

We are interested in identifying the neurobiological principles underlying cognition and decision-making. Using a reductionist approach, we translate psychological questions into the language of neuroscience by distilling them into quantitative behavioral tasks for rodents that enable the monitoring and manipulation of neural circuits mediating cognitive behaviors. We use state-of-the-art electrophysiological techniques to establish the neural correlates of behavior and then use molecular and optogenetic manipulations to systematically dissect their underlying neural circuits.

Given the complexity of animal behavior and the dynamics of neural networks producing it, our studies require quantitative analysis and computational models to guide and sharpen the neurobiological questions. In addition, we started incorporating human psychophysics into our research to validate our behavioral observations in rodents by linking them with analogous behaviors in human subjects.

In terms of topics, our approach is multifaceted. We study (1) the roles of uncertainty in decision-making, (2) the division of labor between different cell types in prefrontal cortex, (3) how the cholinergic system supports sustained attention, and (4) social decisions that rely on shallow, stereotyped circuits. A unifying theme is the use of cell-type and pathway-specific perturbations to effect gain and loss of function for specific behavioral abilities. Through such manipulations of genetically and anatomically defined neuronal elements, we hope to identify fundamental principles of neural circuit function that will be ultimately useful for developing therapies for diseases such as schizophrenia, Alzheimer's dementia, and autism spectrum disorder.

Neural Basis of Decision Confidence

J. Sanders, J. Hirokawa [in collaboration with A. Lak, University of Cambridge; G. Costa and Z.F. Mainen, Champalimaud Neuroscience Program, Portugal]

If you are asked to evaluate your confidence in your decision—how sure are you that you made the correct choice—you can readily answer. What is the neural basis for such judgments? Previously, we discovered neurons in orbitofrontal cortex (OFC) that signal decision confidence, and we are pursuing these observations by trying to establish the causal neural circuit for estimating and acting on decision confidence.

As a first step, we designed a new behavioral task for rats, in which we could measure confidence behaviorally on a trial-by-trial basis. Briefly, rats are trained on a simple olfactory decision task, and by delaying reward, we found that the time they are willing to wait for an uncertain reward is proportional to decision confidence, as predicted by theory. To further refine the role of OFC in confidence, we are trying to "read out" confidence reports, i.e., predict the timing of leaving decisions on a trial-by-trial basis based on neural activity (establish correlation). To demonstrate that OFC is necessary for confidence reports, we used both excitotoxic lesions and pharmacological inactivation techniques and found that confidence reports were disrupted without changing decision accuracy. To establish the sufficiency of OFC for confidence reporting, we are preparing gain-of-function experiments. Because there is no obvious map in the prefrontal cortex, we cannot use electrical microstimulation. Instead, we will use optogenetic activation of neurons defined by projection target using retrograde viruses and attempt to inject extra "uncertainty" into the brain by activating the appropriate population of neurons. Our ultimate aim is to define a precise neural circuit for computing and using decision confidence.

Confidence Judgments in Humans

J. Sanders

In our rodent studies of confidence, we use a computational framework to interpret behavioral and neural data. Nevertheless, we are also interested in understanding the degree to which the theoretical concept of *decision confidence* corresponds to the human notion of subjective confidence. Our goal was therefore to develop a new confidence-reporting task that provides both implicit and explicit measures of decision confidence and is suitable for both rodents and humans in order to make direct

comparisons. Therefore, we tested humans in a paradigm similar to that of rats (using an auditory task) and found that humans follow the same patterns of waiting time and their explicit self-reports of confidence (1–5 scale) correlate with this implicit waiting time measure.

On the basis of these results, we are now in a position to use these quantitative measures of decision confidence to study humans, through collaborations, with neuroimaging and genetic approaches. Moreover, by showing that a single confidence measure is applicable to humans and rodents, our results strengthen the case for using the rat as a model system for studying decision confidence.

Integration of Decision Confidence and Reward Size in Orbitofrontal Cortex

J. Hirokawa

Making optimal decisions requires the integration of different sources of information. For instance, past experience with rewards enables animals to make predictions about their quality, size, and predictability. When the main source of uncertainty is due to a perceptual decision process, estimating decision confidence provides a mechanism for predicting reward outcomes. Can rats combine experienced reward value and decision uncertainty to make optimal choices? And how does the brain dynamically compute and integrate these different estimates to make predictions?

We designed a reward-biased psychometric decision task to dissociate reward estimates based on decision uncertainty and reward history and recorded neurons in the orbitofrontal cortex (OFC). We found that behaviorally, rats optimally combined decision confidence and reward size. In OFC, we observed a heterogeneous representation: one class of neurons signaled decision confidence independent of reward, and another class signaled the integrated negative reward value by combining decision uncertainty with reward size. Therefore, OFC appears to contain a mixture of representations about future outcomes, including separate signals for reward value and decision confidence.

Division of Labor between Distinct Interneuron Subtypes during Behavior

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

The medial prefrontal cortex (mPFC) in humans and rodents has been implicated in a variety of goal-directed behaviors including working memory, inhibitory response control, and attentional set switching. Electrophysiological recordings from mPFC show a great diversity of neuronal responses to specific behavioral variables. On the other hand, we know that the mammalian cortex is composed of a variety of cell types among which GABAergic interneurons exhibit greatest diversity in connectivity, morphology, and intrinsic physiology. Does the anatomical and molecular variety of interneuron subtypes map onto the diversity of neuronal responses in behaving animals? To address this issue, we use optogenetics as a means to identify extracellularly recorded neurons in freely moving mice, focusing on inhibitory interneurons.

We recorded from the two largest and nonoverlapping populations of interneurons: somatostatin (Som) and parvalbumin (Pv) neurons, which synapse onto distal and proximal regions and differentially regulate the input and output of pyramidal neurons, respectively. To engage prefrontal ensembles, we trained mice on a simple reward foraging task in which they ran on a linear track between two platforms, collecting rewards at one platform and triggering rewards at the other platform. Mice slowed their speed during reward zone approach in omission trials, demonstrating that behavioral performance was goal-directed.

We found that Pv and a subtype of Som neurons showed distinct and homogeneous responses during specific behavioral epochs. Furthermore, Pv neurons fired with millisecond synchrony, resulting in brief and precise inhibitory control. In contrast, the asynchronous firing of Som interneurons resulted in weaker and variable inhibitory impact. Our results reveal that two prominent interneuron subtypes previously defined by their connectivity and in vitro physiology comprise complementary networks for inhibitory control in mPFC and point to the possibility that functional response diversity during behavior can in part be explained by cell-type diversity.

Function of VIP Interneurons in Cortical Microcircuits

H.J. Pi [in collaboration with Z.J. Huang, Cold Spring Harbor Laboratory]

We are interested in understanding how the diversity of cortical inhibitory interneurons underlies distinct neural circuit dynamics that directs perception and behavior. As part of this broader project, we have begun to study vasoactive intestinal peptide (VIP)-expressing interneurons. Although VIP neurons constitute less than 2% of total cortical neuronal population, previous studies indicate

that VIP might have a distinct function in local cortical computation. VIP neurons are mostly bipolar and their processes are vertically oriented. Interestingly, these neurons specifically target other inhibitory neurons and avoid pyramidal cells. On the basis of these ideas, we are currently testing the hypothesis that VIP inhibitory interneurons provide local excitation to a patch of cortex via disinhibition of other interneurons.

Neural Representation of Social Decisions and Rewards

E. Demir [in collaboration with R. Axel, Columbia University]

Mice, like humans, are social animals. To interact, cooperate, and compete with others, mice have to collect information about each others' identity, fertility, and likely intent. We would like to understand how social information is represented, computed, and used by mice. In rodents, a main source of information for social decision-making and reward valuation is the chemosensory system. The neural circuits supporting these tend to be shallow, from sensory input to motor actions, and highly stereotyped, enabling the systematic dissection of this system.

We have begun our studies by recording from the medial amygdala, the first brain region that combines information from the two olfactory systems, the main olfactory system transducing volatile odors and the vomeronasal system detecting behaviorally significant chemical cues, such as pheromones. Currently, we are developing psychophysical social behavior tasks to use for our electrophysiology studies. To deliver social stimuli and determine their "value" for a mouse, we began to develop a new behavioral setup, the "mouse carousel." This system improves stimulus delivery precision and repertoire. To characterize the social cue responses (e.g., urinary proteins) of medial amygdala neurons, we also began to develop protein ports to systematically deliver pheromonal stimuli. Currently, we are performing optogenetic experiments to map the behavioral relevance of social information onto specific medial amygdala neurons.

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

B. Hangya, S. Ranade, M. Faleiro

The cholinergic basal forebrain (CBF) is a vitally important yet poorly understood neuromodulatory system that is thought to have significant roles in cognitive functions, including attention. Projections of CBF innervate the entire cortical mantle and release acetylcholine, which enhances cortical processing. Our goal is to understand the function of the CBF in sustained attention, using a combination of molecular genetic, electrophysiological, optogenetic, and psychophysical techniques.

We use a knockin mouse line (Chat-cre) to specifically target cholinergic neurons in the basal forebrain. We can express optical activators/inactivators delivered via viral vectors specifically in these neurons to manipulate their activity with high spatiotemporal precision. We have successfully developed visual and auditory versions of sustained attention tasks in mice. In these tasks, mice report target stimuli occurring at uncertain times. Performance and reaction time are used as an indicator of attention. We manipulate temporal expectancy by using specific distributions for stimulus timing, thus modulating attention in a temporally precise, graded manner, which is reflected by the reaction times of the animals. We are currently testing the causal role of acetylcholine in this task by transiently inactivating cholinergic neurons with light during behavior. We have also started recording from identified cholinergic neurons to assess their role in sustained auditory attention. Using these tools enables an unprecedented degree of mechanistic investigation of the behavioral functions of the CBF system and provides an entry point for future studies of cholinergic degenerative diseases, such as Alzheimer's disease.

Mechanisms of Cholinergic Modulation in Auditory Cortex

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

Acetylcholine (Ach) has profound physiological effects on cortical neurons. Cholinergic agonists increase firing rates, decrease firing correlations between neighboring neurons, and change the receptive field properties of cortical neurons.

What is the physiological mechanism by which Ach modulates cortical neuronal firing? The goal of this project is to investigate the cellular and circuit mechanisms of cholinergic modulation of spontaneous and stimulus-evoked activity of cortical neurons in the auditory cortex (ACx). We targeted the light-activator, ChR2, to cholinergic neurons projecting to ACx to evoke light-triggered release of acetylcholine. Using this technique, we observed the effects of acetylcholine on auditory responses. A high percentage of neurons in-

creased their firing rate in response to light activation and some also showed changes in their auditory tuning curves. Using cell-attached recordings, we now plan to investigate the underlying cellular mechanism of cholinergic modulation.

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Adam Kepecs (*fourth from left, front*) and lab members

THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

A.A. Koulakov

Our laboratory develops mathematical models of neural processing. We work in parallel on three important topics. First, we are attempting to formulate an accurate mathematical model for combining genetic information and experience (nature and nurture) during the formation of connections between neurons. 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 elusive concept that excites the imagination of chemists, neuroscientists, and experimental psychologists alike. Using this 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 have developed a robust description of the human perceptual olfactory space. This description is analogous to understanding the main directions in the human color space, i.e., red, green, and blue. These main perceptual dimensions can be understood in terms of 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 hippocampus. These models 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 drug therapies.

A Race Model for Singular Olfactory Receptor

A.A. Koulakov [in collaboration with I. Iossifov and B.E. Kolterman, Cold Spring Harbor Laboratory]

In vertebrates, olfactory sensory neurons choose only one olfactory receptor to produce out of ~2000 possibilities. This feature is important because it lowers the detection threshold of odorants by olfactory receptors. The mechanism for this singular receptor expression is unknown. Here, we study a computational model that can stochastically select a single gene out of a large number of possibilities. In this model, receptor genes compete for a limited pool of transcription factors (TFs). The gene that recruits a target number of TFs is selected for expression. To support this mechanism, we have attempted to detect repeated motifs within known sequences of mouse olfactory receptor promoters. Motifs were found that are significantly overrepresented in olfactory versus other gene promoters. We identified possible TFs that can target these motifs. Our model suggests that a small number of TFs can control the selection of a single gene out of ~2000 possibilities.

An Exactly Solvable Model of Stochastic Cre-Lox Recombinations

A.A. Koulakov [in collaboration with Y. Wei, Cold Spring Harbor Laboratory]

The Cre-Lox system is used as a genetic tool to control site-specific recombination events in genomic DNA. If multiple recombination sites are organized in a compact cluster within the same genome, a series of stochastic recombination events may generate substantial cell-specific genomic diversity. This diversity is used, for example, to distinguish neurons in the brain of the same multicellular mosaic organism, within the brainbow approach to the neuronal connectome. Thus, every

cell in an organism could be identified on the basis of a relatively short unique DNA barcode. Here, we study an exactly solvable statistical model for site-specific recombination operating on a cluster of recombination sites. We consider two types of recombination events: inversions and excisions. Both of these events are available in the Cre-Lox system. We derive three properties of the sequences generated by multiple recombination events. First, we describe the set of sequences that can in principle be generated by multiple inversions operating on the given initial sequence. Second, we demonstrate that after a large number of stochastic inversions, every sequence that can be generated is generated with equal probability. Finally, we derive equations for the probability of finding a sequence as a function of time in the limit when excisions are much less frequent than inversions, such as in shufflon sequences.

Competition Is a Driving Force in Topographic Mapping

A.A. Koulakov [in collaboration with C. Pfeiffenberger, J.W. Triplett, J. Yamada, B.K. Stafford, D. Feldheim, University 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 show that topographic maps are substantially distorted under conditions of reduced competition (Fig. 1). RGC axons project almost entirely to the anteromedial 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.

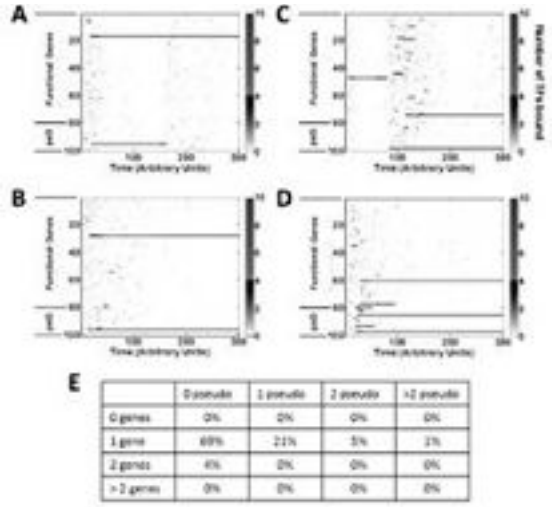

Figure 1. A race model for singular olfactory receptor (OR) expression. (*A–D*) Number of TFs bound to each promoter as a function of time for 80 functional genes and 20 pseudogenes (psGs) with cooperative binding of TFs. Each panel shows a simulation for a single neuron. Pseudogenes do not invoke a negative feedback mechanism to stabilize gene choice, whereas functional genes do. OR expression occurs when at least four TFs are bound. Singular expression occurs for functional genes in almost all cases, with occasional coexpression of pseudogenes (*B–D*). (*E*) Table of probabilities for various psG and functional gene expression level combinations determined from 1000 simulated cells.

The Structure of Human Olfactory Space of Mixtures

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

We investigated the responses of human observers contained in the atlas of human character profiles (AOCP) by Andrew Dravnieks. We had shown previously that 146-dimensional (146D) profiles of odorants that represent the responses of human observers to a variety of monomolecular odorants can be accurately described by a two-dimensional (2D) curved manifold. Here, we investigate the responses of human subjects to the set of 15 mixtures contained in the same database. The particular question that we address is whether the percepts of mixtures (the mixture space) reside close to the olfactory space determined from the monomolecular odorants. We show that indeed the mixture space can be predicted by the percepts of monomolecular odorants (Fig. 2). The important difference, however, is that the mixture space contains one extra dimension compared to monomolecular odorants. Thus, approximating 146D profiles of monomolecular odorants with the 2D curved manifold allowed us to include 51% of variance

Figure 2. The structure of human olfactory space. The odors (crosses) cluster around a 2D curved surface in the perceptual space. The 2D surface exists in a 146D 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.

contained in the data. In the case of mixtures, the same amount of variance can be explained by a 3D curved space. One extra dimension, however, is predicted by the small residual fluctuations of monomolecular percepts around the 2D curved monomolecular space. We conclude that the responses of human observers to mixtures can be found to be low dimensional and predictable from the percepts of monomolecular odorants.

Sparse Incomplete Representations: A Novel Role for Olfactory Granule Cells

A.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 that 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. It can be argued 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 and 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.

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

B. Li S. Ahrens S. Ghosh M. Penzo
H. Chae H. Li Z. Perova
K. Delevich R. Paik M. Wang

We are interested in understanding the synaptic mechanisms of behaviors that are related to 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 these 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 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 cellular basis of depressive disorders is poorly understood. Recent studies in monkeys indicate that neurons in the lateral habenula (LHb), a nucleus that mediates communication between forebrain and midbrain structures, can increase their activity when an animal fails to receive an expected positive reward or receives a stimulus that predicts aversive conditions (i.e., disappointment or anticipation of a negative outcome). LHb neurons project to and modulate dopamine-rich regions such as the ventral-tegmental area (VTA) that control reward-seeking behavior and participate in depressive disorders. Our study shows in two learned helplessness models of depression that excitatory synapses onto LHb neurons projecting to the VTA are potentiated. Synaptic potentiation correlates with an animal's helplessness behavior and is due to an enhanced presynaptic release probability. Depleting transmitter release by repeated electrical stimulation of LHb afferents, using a protocol that can be effective on depressed patients, dramatically suppresses synaptic drive onto VTA-projecting LHb neurons in brain slices and can significantly reduce learned helplessness behavior in rats. Our results indicate that increased presynaptic action onto LHb neurons contributes to the rodent learned helplessness model of depression.

We are currently determining the specific pathways/mechanisms that can mediate the behavioral effects of DBS (deep brain stimulation) in LHb. We are also using optogenetic methods to either manipulate LHb cellular activity or modulate the monoaminergic inputs to LHb, and to determine the behavioral effects.

Depression and Resilience: Role of the Medial Prefrontal Cortex

We are 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 45:* 365 [2005]). Aberrant activity of the mPFC also accompanies clinical depression, and DBS in mPFC leads to remission of treatment-resistant depression (Mayberg et al., *Neuron 45:* 651 [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.

To determine cellular activity in the mPFC in different groups of animals, we measured c-Fos expression in the infralimbic (IL) and prelimbic (PL) regions of the mPFC. We found that in the learned helplessness (LH) model of depression, neurons in the mPFC were activated. After the LH procedure, animal behavior diverged to become either depressed or resilient to depression. Interestingly, the resilient animals had significantly enhanced neuronal activation compared with the depressed

animals, indicating an active coping process. We are currently examining whether deferential plastic changes in the synapses onto mPFC neurons in response to stress are critical in determining 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 at attaining a more complete understanding of the synaptic and circuitry mechanisms underlying depression.

Genetic Deficiency, Dysfunction of the Thalamic Reticular Nucleus, and Schizophrenia

The thalamic reticular nucleus (TRN) integrates excitatory inputs from the cortex and thalamus, and sends inhibitory outputs to control thalamic function. This cortico-TRN-thalamic circuitry is thought to modulate the processing of sensory stimuli and to have an important role in cognitive functions such as attention. Dysfunction of TRN has been implicated in schizophrenia, a complex mental disorder in which impairments in cognitive functions, including attention, are core symptoms; however, the molecular and cellular mechanisms underlying TRN circuit dysfunction in schizophrenia are not well understood.

Recent studies indicate that molecules linked to schizophrenia are enriched in TRN neurons. These include ErbB4 and its ligand neuregulin-1 (NRG1). The NRG1/ErbB4 signaling pathway has been implicated in many biological processes, including the development and function of synapses in the central nervous system. A plausible hypothesis, therefore, is that deficits in NRG1/ErbB4 function can alter the formation or function of synapses that assemble the TRN circuitry, leading to circuit dysfunction and cognitive impairments associated with schizophrenia. Consistent with this idea, recent results from my lab indicate that reducing ErbB4 protein levels in TRN neurons causes alterations in attention in mice (Fig. 1).

We focus on a class of TRN neurons that express somatostatin (SOM^+) and constitute a major cell popu-

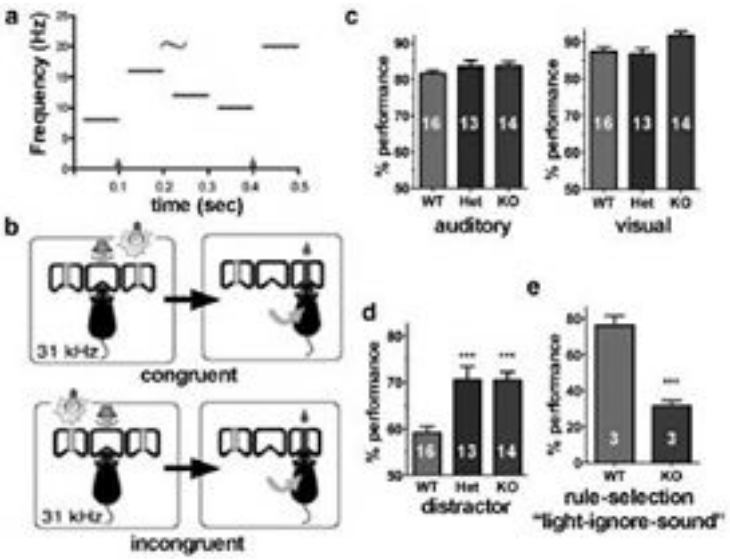

Figure 1. Deletion of ErbB4 in SOM^+ TRN neurons altered attention. We first trained mice in the auditory or visual two-alternative choice task (2-AC). In the auditory 2-AC, mice were rewarded for correctly discriminating a target sound. The tone frequency indicated the location of reward (right or left). Animals were further trained in a visual 2-AC, in which a left or right light signaled reward in the left or right, respectively. Having learned these basic 2-AC tasks, mice were tested in a "distractor" task and a "rule-selection" task to examine goal-directed attention. (*a*) Schematic diagram of the "distractor" task, in which the target tone (gray "~") is embedded in a stream of distractor tones (black lines). (*b*) Schematic diagram of the "rule-selection" task, in which the mice were presented with both the sound and light. However, only one of the stimuli predicted reward. For example, in a "light-ignore-sound" session (as shown), only sound predicts reward, whereas light is random to reward. (*c*) All groups can learn the basic auditory (*left*) or visual (*right*) 2-AC task. (*d*) $SOM/ErbB4^{+/-}$ (Het) and $SOM/ErbB4^{-/-}$ (KO) mice had enhanced performance in the "distractor" task. (*e*) KO mice had impaired performance in the "rule-selection" task. Data are presented as mean ± S.E.M.; $^{***}p < 0.001$; numbers in the bar indicate *N*.

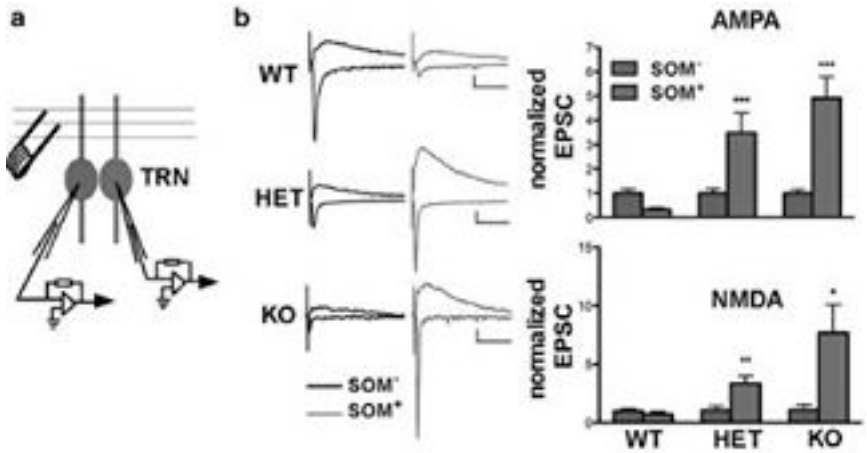

Figure 2. Regulation of excitatory synapse in SOM^+ TRN neurons by ErbB4. (*a*) A schematic diagram of the recording configuration. (*Right*) A SOM^+ neuron. (*b*, *Left*) Sample EPSC traces recorded at +40 and −70 mV holding potentials, from ErbB4 wild-type (WT), SOM/ErbB4$^{+/-}$ (HET), and SOM/ErbB4$^{-/-}$ (KO) mice (calibrations: 20 pA and 50 msec). (*b*, *Right*) Quantification of AMPA and NMDA EPSC amplitude normalized to the mean of SOM^- neurons ($^*p < 0.05$; $^{**}p < 0.001$; $^{***}p < 0.0001$).

lation in the TRN. Our preliminary results indicate that ErbB4 is enriched in SOM^+ neurons in the TRN and that it has a rather unexpected role: It suppresses glutamatergic synaptic transmission, an effect that deviates from its role in other brain areas, where ErbB4 positively regulates synaptic function (Fig. 2). We are capitalizing on these exciting preliminary findings and are determining whether and how ErbB4 controls the synaptic connectivity of SOM^+ TRN neurons, thereby regulating the cortico-TRN-thalamic circuitry function. To achieve these goals, we use a combination of genetic, molecular, optogenetic, and electrophysiological methodologies. Results from this research will shed light on the cellular mechanisms underlying TRN circuit dysfunction in major psychiatric disorders.

PUBLICATION

Li B, Piriz J, Mirrione M, Chung C, Proulx C, Schulz D, Henn F, Malinow R. 2011. Synaptic potentiation onto habenula neurons in the learned helplessness model of depression. *Nature* **470:** 535–539.

MECHANISMS OF DEPRESSION

F. Henn M. Mirrione
N. Ruth

Our research is aimed at understanding the neural basis of psychiatric disease, with an emphasis on depression. The focus of our current work involves the use of a unique animal model of depression that we have developed over the last decade to understand the anatomy and pathophysiology of depression. We use a model involving uncontrollable and unpredictable stress, which leads to "learned helplessness" in ~15%–20% of animals. We conducted outbreeding studies using the most helpless animals to form one strain and the animals most resistant to this stressor to form the other strain. A comparison of these two strains using positron emission tomography (PET) carried out at Brookhaven National Laboratory suggested to us that the largest difference in activity in the brains of the two strains was in a small structure, the lateral habenula that sits between the cortex and the midbrain aminergic nuclei (see Fig. 1).

Working with Bo Li at CSHL, we were able to show that the l. habenula is overactive in helpless animals, leading to an inhibition of dopamine release in reward centers. In addition, our PET data suggest that the l. habenula also leads to serotonin decreases in the helpless state, which fits with the idea behind most antidepressant medications that attempt to increase serotonin. The driver of this activity appears to be the medial prefrontal cortex (mPFC), which also seems to be abnormal in the helpless animals. Thus, we looked at the mPFC in our helpless animals in collaboration with a group at the University of California, Irvine, headed by Gary Lynch and found that there are 40% fewer synapses in the helpless cortex than in the resilient animals, a result that is consistent with the functional behavior of the strains and appears to reflect pathology due to increased glutamate extracellularly leading to synaptic damage. This is consistent with the result in patients, where ketamine, which restores glutaminergic synapses, reverses many cases of treatment-resistant depression. Unpublished work, done at our suggestion, shows down-regulation of habenular activity in patients following ketamine administration. Our earlier data suggest that this decrease in synaptic boutons is due to decreased glutamate transporters in astrocytes in the helpless animals. We just received a patent for a class of drugs that up-regulate the astrocytic glutamate transporter as possible antidepressants, and we are arranging to test this idea at the National Institutes of Health (NIH) clinical center in the coming year.

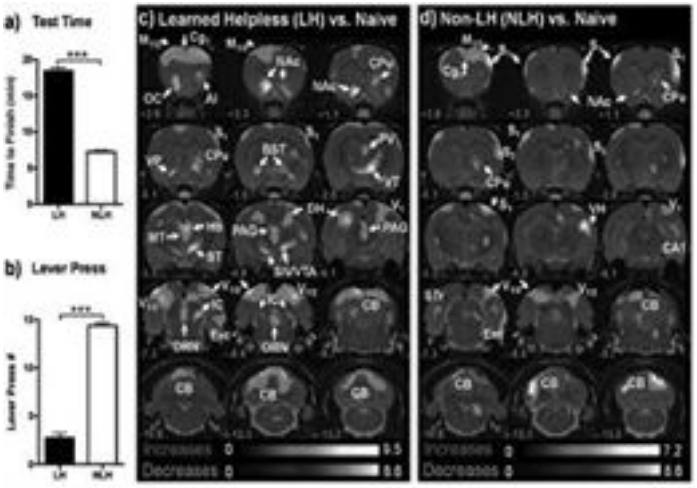

Figure 1. PET scans of helpless and nonhelpless lines versus naïve rat brains. Note habenula activation (Hb) in helpless line but not in resistant line.

We are continuing to look at the details of the anatomy between the two strains using c-*fos* to mark activated neurons and specific stains to identify the transmitters used by these cells. Under the direction of Dr. Mirrione, we are developing a whole-brain atlas of both the helpless line and resilient lines to determine the transmitters and circuits that underlie the differences.

We are also looking at the whole genome sequences of the two lines in collaboration with Professor McCombie at CSHL to get an idea of the genetic differences in the lines as a clue to genetic factors in human depression.

Much of the work in the past 2 years was aimed at defining the circuit that mediates depression; one test of the central hypothesis that the l. habenula is a key structure mediating this circuit is to use deep-brain stimulation to inhibit the overactive l. habenula in treatment-resistant cases of depression. One trial case, done a few years ago in Europe at my former institute, resulted in the total cure of the patient. We have applied to the Food and Drug Administration (FDA) in the United States to carry out a large trial of this procedure.We have worked with a neurosurgeon and psychiatrists at Mount Sinai Medical Center to develop a team to carry out these studies and have submitted a request to the FDA.We should be able to begin in the next year to definitively test this hypothesis.

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Fritz Henn, Martine Mirrione, Nora Ruth, Bo Li

INTEGRATIVE SYSTEMS NEUROBIOLOGY

P. Mitra N. Franciotti, P. Grange, G. Havkin, N. Jakimo, D. Johnston, J. Jones, S. Mukhopadhyay, J. Novy, V. Pinskiy, C. Powell, A. Tolpygo, K. Weber, L. Wood, 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 are now undertaking a major in-house experimental project to generate the first brainwide mesoscale connectivity map in the mouse.

Continuing in the Mitra laboratory in 2011 are Pascal Grange and Swagatam Mukhopadhyay (postdoctoral fellows), Jamie Jones, Alex Tolpygo, and Joshua Novy (laboratory technicians), Sandra Michelsen (administrator), Vadim Pinskiy and Zhen Gong (graduate students), Gregor Havkin (informatics manager), Linzie Wood (project assistant), and Caizhi Wu (research associate). Kevin Weber, Neil Franciotti, and Caitlin Powell (laboratory technicians) and Noah Jakimo (informatics analyst) joined this past year.

We also have close collaborative ties with multiple research groups at CSHL and other institutions, which currently include Dr. Nicholas D. Schiff at the Weill Medical School of Cornell University, where Dr. Mitra is an adjunct associate professor; a laboratory evolution study in *Drosophila* with Josh Dubnau at CSHL; the Brain Architecture Project, with collaborators at multiple institutions, notably Harvey Karten (University of California, San Diego), Kathy Rockland (Massachusetts Institute of Technology), Michael Hawrylycz (Allen Institute of Brain Research), and Jin Hyung Lee (University of California, Los Angeles); a collaboration with Josh Huang at CSHL on GABAergic interneuron expression in ASD mouse models; and a collaboration with Gregory Hannon at CSHL on analysis of a small hairpin RNA (shRNA) screen.

Mouse Brain Architecture Project

V. Pinskiy, A. Tolpygo, C. Wu, N. Jakimo, J. Jones, K. Weber, J. Novy, G. Havkin, P. Grange

Brain function is determined by its circuitry, but our knowledge of mammalian brain connectivity is highly incomplete. We have set out to address this problem in the Mouse Brain Architecture Project, in which we are comprehensively determining the patterns of neural connectivity between brain regions.

Following initial funding by the Keck foundation and support from CSHL, we obtained two National Institutes of Health (NIH) grants (a Transformative-R01 and RC1 Challenge Grant) to produce the connectivity map of C57BL/6J mice and to align these results to the Allen Brain Atlas. Our plan involves injecting anterograde (BDA, AAV) and retrograde (CTB, Rabies) tracers systematically on a grid of locations distributed throughout the brain. Each injection site yields information about the other regions of the brain projecting to and from the injected region. This project is now in full production phase. The pipeline to process tissue from tracer-injected mice through to image analysis is fully functional and all equipment has been integrated into a laboratory information management system (LIMS).

We have already injected more than 800 production animals at ~200 different sites. We are currently performing cortical injections in columnar fashion. For adeno-associated virus (AAV) injections, we aim to simultaneously label superficial (layers 1–3) and deep layers (1–4), in two colors, green fluorescent protein (GFP) and red (TdTomato). These injections are more elaborate than single-point injections (used to target subcortical structures), but we are able to perform them at the same

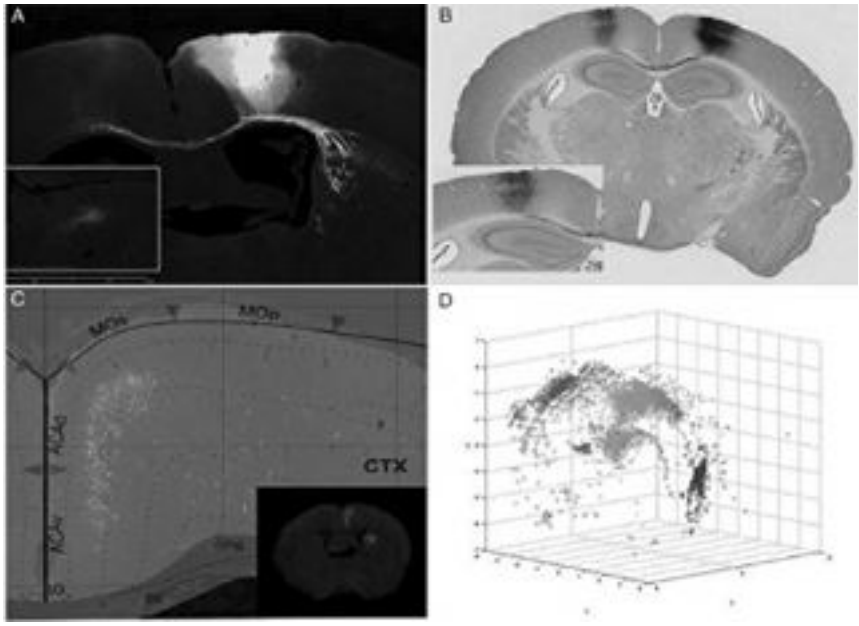

Figure 1. MBA pipeline output and examples of data generated from the MBA pipeline. (*A*) Cortical AAV fluorescent injections performed at adjacent sites. Contralateral labeling via callosal projections is evident. (*Inset*) Thalamic projections observed in the same brain. (*B*) Cortical BDA injection showing contralateral projection through the corpus callosum. (*Inset*) Close-up of contralateral projection area. (*C*) Images containing labeled cells are registered with the Allen Reference Atlas. (*D*) Specially designed software automatically determines the locations of labeled cells and plots them in three-dimensional space.

throughput and at the same quality, due to the versatility of the stereotaxic method, designed the previous year. Computer-guided injections are the standard in the laboratory and are being used for all cortical injections. Figures 1A,B are examples of cortical injections. Figures 1C,D show the computational processing of the image data registration of the sections onto the Allen Reference Atlas (ARA) and the automatic detection of labeled cells.

During the last year, we have begun the process of analyzing the collected data and making it available on the MBA portal. We have developed an interactive data browser that allows for high-resolution viewing of the data in a neuroanatomical context. The user is able to view all sections of a given brain, adjust individual viewing parameters (color channels for fluorescence), zoom and pan throughout the section, provide regional annotations, and link to appropriate neuroanatomical references, such as ARA and BAMS (Brain Architecture Management System, University of California, Los Angeles). We have worked extensively to improve the performance of this server, such that many users can simultaneously access the data without delay. Figure 2 shows the graphical user interface of the portal.

In the last year, we have also begun collecting auxiliary data sets for the portal, using the MBA pipeline. These data sets include a complete set of nissl-stained brains (sectioned in the coronal, sagittal, and transverse planes), immunohistochemical staining of various common neuronal markers (lectin, TH, PV, etc.), and myelin. Myelin has been one of the most challenging to gather; results are shown in Figure 3.

Finally, this project has also benefited from a summer course that Dr. Mitra cofounded at CSHL in 2010 and held again in 2011.

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 at http://brainarchitecture.org.

Alterations in Brain-Wide GABAergic Neuroanatomy in ASD Mouse Models

S. Mukhopadhyay, V. Pinskiy, A. Tolpygo, J. Jones, N. Franciotti, K. Weber, J. Novy, Z. Collins

In collaborative work with Josh Huang at CSHL, we have received funding from the Simons Foundation Autism

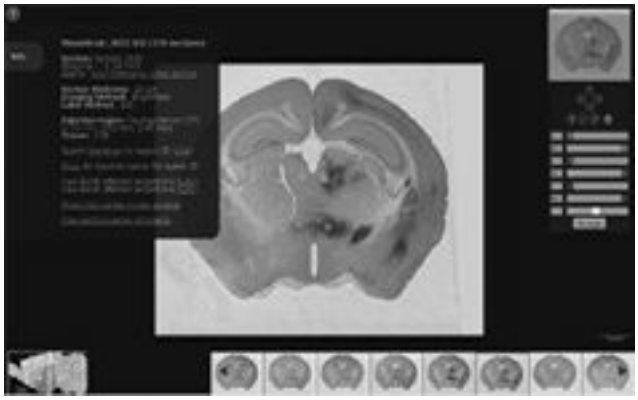

Figure 2. Project website interactive interface. Data sets generated by the MBA pipeline are included in an interactive database. The web-based interface allows for viewing of high-resolution images of brain slices as well as an information panel that includes metadata and links to related resources.

Research Initiative (SFARI) to generate brain-wide maps of inhibitory neuron subpopulations and inhibitory long-range projections in both wild-type (WT) mice and autism spectrum disorder (ASD) model mice to determine the neuroanatomy of dysfunctional inhibition relevant to autism. This project uses the MBA pipeline to process the mouse models developed by Josh Huang's group. The project focuses on four GABAergic neuron subtypes: PV (parvalbumin), SOM (somatostatin), CRH (corticotrophin releasing hormone), and VIP (vasointestinal peptide). The project was started in the summer of 2011. Since then, we have processed an $N = 2$ of each subtype for 56-day-old animals. As part of the grant, we are also studying the development of these mouse models, by processing animals at p14 and p36. As part of this project, we have developed an algorithm to allow for the automated counting of cells across the whole brain. An example of the cell-counting algorithm is shown in Figure 4.

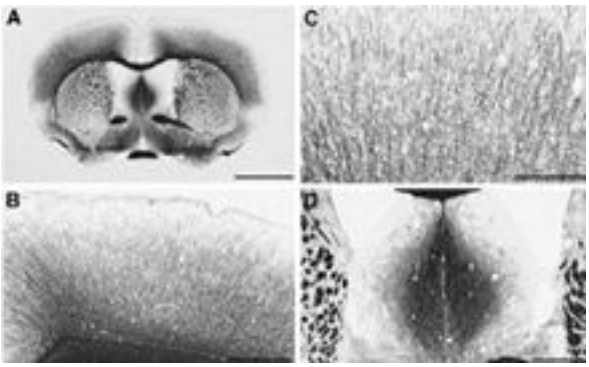

Figure 3. Whole brain myelin staining. As part of the MBA project, we have initiated an effort to collect auxiliary data sets of common biomarkers, including myelin. We have successfully modified the MBA pipeline to produce high-quality myelin staining of 20-μm mouse brain sections. Bars, 2 mm and 500 μm (*B–D*).

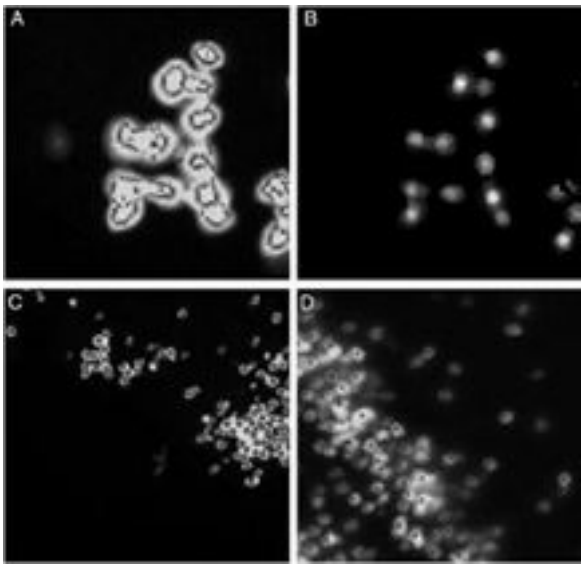

Figure 4. To allow for an efficient and reliable means of counting labeled neurons throughout the large data stacks, we developed an algorithm that takes advantage of the approximate symmetries of objects and uses multiple features of the objects to determine their centers in an iterative method. In the specific implementation, we used the edge-detected-image pixels (*A*) and the gradient of intensities on these pixels as the features. Intensity gradient direction of edge pixels, weighted by the angle between points, is used to determine the approximate center of each cell (*B*). After further iteration of the algorithm (sharpening) and thresholding, the center of the cells is detected (*C,D*). This algorithm is appropriate for cell counting even in seemingly dense regions and in regions where the cells have uneven intensities (a different vertical position within the 20-μm section).

Mapping Long-Range Neuronal Circuits in the Postmortem Human Brain

V. Pinskiy, C. Powell

Our goal is to develop a practical method of studying long-range connectivity in postmortem human tissue.

To this end, we continue to work on a method that allows for the accelerated transport of DiI through postmortem tissue while maintaining tissue integrity. We have also been exploring methods of imaging whole human brains and are in the process of purchasing a large-format, whole-slide imaging scanner.

We have verified that an electrical field of adequate strength can be applied and sustained across a tissue sample using our current "tractophoresis" prototype. We have been reengineering the current apparatus to allow for prolonged electrical field applications while minimizing damage to the tissue over time. The use of a dual-phase gel construct has allowed for extended runs at relatively stable sustained currents, as compared to our previous all-agarose design. Originally, we were only able to run the system for a maximum of 6–7 h at ~10 V/cm before contact between tissue and gel eroded at the positive terminal to the point that the gel needed to be recast. With the addition of a polyacrylamide layer, samples can be run routinely under a similar electrical field magnitude for 24–48 h with little change in sustained current through the system being observed; we have even achieved a run of more than 100 h with this design.

Maintaining tissue integrity remains as the main limiting factor in long-term sustained electrical field application. We are testing the use of different mineral oil formulations to mitigate dehydration of the sample during the application of the electrical field. We are continuing to test and perfect our methodology using sheep brains.

We have also begun the process of developing practical methods of sectioning and imaging whole human brains. We are running initial trials with whole sheep brains. Although we optimize our methods for DiI propagation, we intend to proceed with traditional histochemical processing of whole brain slides, beginning with myelin-staining studies. We also plan to configure the soon-to-be acquired TISSUEscope large-format slide scanner for the imaging of existing neuroanatomical samples from the collection of Dr. Patrick Hof. This project is supported by the G. Harold and Leila Y. Mathers Charitable Foundation.

Analysis of Gene Expression in the Mouse Brain

P. Grange

Statistical analysis of coexpression of genes. Large sets of genes related to addiction have been compiled in public databases. It is desirable to establish the coexpression properties of such sets of genes in order to prioritize further studies. We developed probabilistic techniques to compare the coexpression properties of sets of genes to those of random sets of genes. These techniques were applied to extract highly coexpressed genes from a set of 288 genes from the NICSNP database (single-nucleotide polymorphisms [SNPs] associated with nicotine addiction), using the Allen Gene Expression Atlas (AGEA) of the mouse brain. This research was supported by NIDA (National Institute on Drug Abuse). The application of these methods to the human data released by the Allen Institute is in progress. We established a collaboration with Sharmila Basu (MindSpec) and analyzed a set of 26 genes related to autism, which was found to have higher coexpression compared to a random set of genes. These studies indicate that the AGEA coexpression data can be useful in prioritizing gene lists obtained from human genetic studies and having insight into intervening phenotypic variables.

Brainwide distribution of cell types. The AGEA corresponds to the *gene-based* approach to the study of gene expression in the brain: Expression of a single gene is measured using in situ hybridization in the whole brain. The results cover the whole brain, but they have no cell-type specificity. In contrast, a cell-based approach involves the isolation of homogeneous neuronal populations or even single cells. This approach is cell-type-specific but does not cover the whole brain. We obtained microarray data for 64 different cell types from Sacha Nelson (Brandeis University). We proposed a linear model decomposing the gene expression in the AGEA as a sum over cell-type data. This allowed us to estimate the spatial density of cells of a given type, thus combining the complete brain coverage of the AGEA and the cell-type specificity of microarray data.

Evolutionary ages of genes and neuroanatomy. We used the OMA ortholog data set to infer the evolution of mouse–brain neuroanatomy from the standpoint of the evolution of genes. For each gene in the AGEA Atlas, using the phylogenetic tree for all fully sequenced species and the presence of orthologs of the gene in these species, we constructed a discrete measure of "evolutionary age." We found sets of genes appearing at 12 major branching points in the phylogenetic tree. We studied the deviation of these dated sets of genes from the average expression across the whole AGEA. Some of these deviations are statistically significant and region-specific: For instance, the genes dated to the appearance of vertebrates are overly expressed in the cerebellum.

shRNA Library Analysis

S. Mukhopadhyay

In collaboration with Greg Hannon at CSHL, we have been developing machine learning tools and models inspired by population genetics to study an experimental selection process: a massively parallel sensor assay to extract potent shRNA. The observed success of shRNAs in this selection process was used to construct a machine learning tool (Random Forest and Boosted Trees) to predict shRNAs that are potent in vivo. The predictions are being tested in the lab.

PUBLICATION

In Press

Helmstaedter M, Mitra PP. 2012. Computational methods and challenges for large-scale circuit mapping. *Curr Opin Neurobiol* **22:** 162–169.

(*Left to right*) Partha Mitra, Noah Jakimo, Douglas Johnston

IDENTIFICATION OF DISRUPTED BRAIN CIRCUITS IN MOUSE MODELS OF AUTISM AND SCHIZOPHRENIA

P. Osten Y. Bao, Y. Kim, R. Palaniswamy, N. Takada, J. Taranda, K.U. Venkataraju

Serial Two-Photon Tomography: An Automated Brain-Wide Screening of Neural Circuits in Wild-Type Mice and Genetic Mouse Models of Autism and Schizophrenia

Our laboratory is focused on the study of brain circuits mediating complex behaviors, such as different forms of social behaviors, and how these circuits may be disrupted in genetic mouse models of autism and schizophrenia. The use of serial two-photon (STP) tomography, a novel whole-brain microscopy method (Ragan et al. 2012), allows us, for the first time, to perform brain-wide and cellular resolution screening of neural activation evoked during behavioral tasks (Fig. 1). The STP technology is matched by a pipeline of custom-built computational and image-processing methods that serve to reconstruct the brain-wide data sets and statistically compare the detected brain-activation patterns within a standard mouse brain atlas. The identified brain regions of activation are interpreted as candidate "nodes" of the brain circuit mediating the behavior being investigated, and the mean number of activated cells per node is taken as a measure of the strength of brain activation. The validity of the circuit nodes in mediating the behavior is tested by pharmacologically "removing" one or more nodes (e.g., by using stereotaxic viral vector-based delivery of DREADD molecules) and measuring the effect of the manipulation on the animal's behavioral performance. Finally, the connectivity between the nodes is determined by retro- and anterograde tracing in order to generate complete diagrams of mouse brain circuits mediating specific behaviors.

The Application of STP Tomography to the Study of Autism and Schizophrenia in Genetic Mouse Models

The goal of this work is to identify brain-circuit-based endophenotypes related to autism and schizophrenia in genetic mouse models, which could be used in preclinical research in the future. The use of STP tomography allows us to rapidly screen neural activation in the whole mouse brain at cellular resolution in order to identify anatomical regions with altered functions. Such regions are interpreted as "nodes" of candidate brain circuits affected in the mouse model, and the cellular and molecular pathophysiologies underlying the region-specific changes are studied by traditional methods of experimental neuroscience. These follow-up studies aim to generate hypotheses for the development of therapeutic agents that could eliminate the abnormality and restore normal circuit functions in the mouse model. Our approach thus aims to provide a comprehensive platform of quantitative and standardized brain-circuit-based assays that could be used to identify autism-related pathophysiologies and test novel treatments in animal models. We currently focus on five mouse models of autism: 16p11.2 deletion and duplication mice, which replicate one of the most frequent and penetrant mutations associated with autism (the 16p11.2 copy-number variation in autism was identified by Mike Wigler's lab and the mice were generated by Alea Mills' lab, both at CSHL), and Neuroligin R451C, Neuroligin 4 knockout, and CNTNAP2/Caspr2 knockout mice, which carry mutations in genes with synaptic functions.

The Application of STP Tomography to the Study of GABAergic Neurons in the Mouse Brain

The goal of this work is to generate a quantitative atlas of GABAergic neurons, the most diverse class of cells in the mammalian brain, which would provide the first comprehensive model of brainwide GABAergic circuitry and its role in brain function (Fig. 2) (this work is a collaboration with Josh Huang's lab at CSHL). GABAergic neurons maintain the delicate excitatory-inhibitory balance in the brain, which enables persistent activity while avoiding seizures, control synaptic integration and spik-

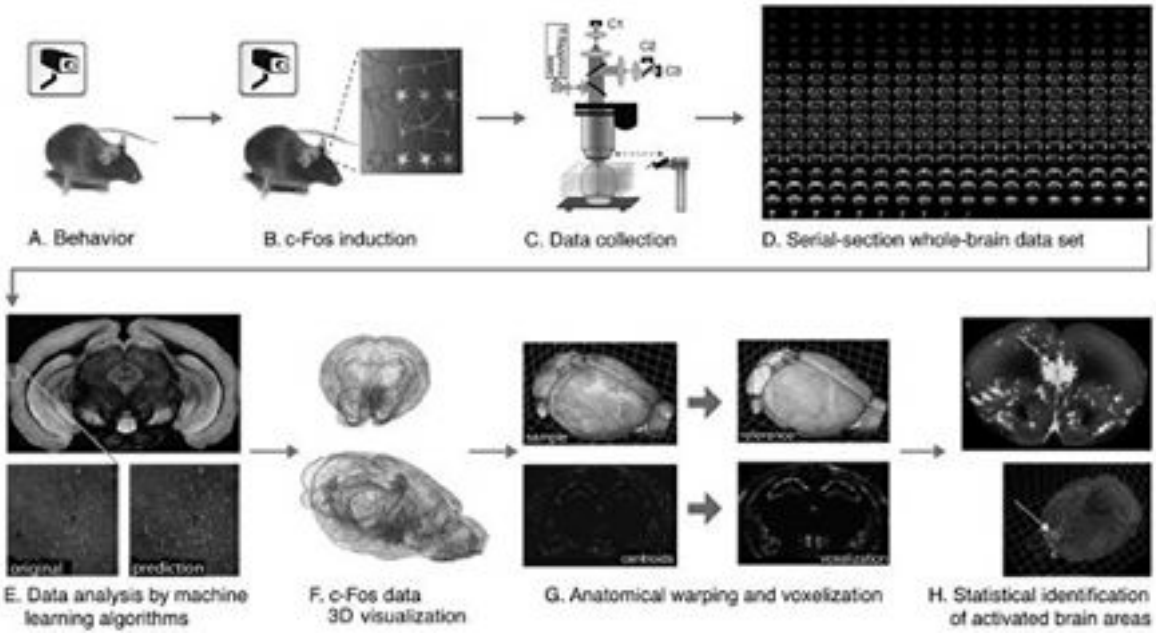

Figure 1. Brain-wide screen of behavior-evoked neural activation. (*A–D*) At the end of a behavioral task, the brain of a transgenic c-Fos-GFP (green fluorescent protein) mouse is imaged by STP tomography. (*E–F*) c-Fos-GFP-positive cells are computationally detected and reconstructed. (*G*) The data sets are warped onto a standard "reference" brain (*top panels*) and voxelized for statistical comparisons (*bottom panels*). (*H*) c-Fos-GFP distribution in control and experimental brains is compared by series of negative binomial regressions, with a false discovery rate of 0.01. The arrows point to an example of a strong, behavior-evoked activation in the medial prefrontal cortex.

ing of excitatory neurons, and generate network oscillations that serve as temporal structures for orchestrating brain-wide neural ensembles. It is therefore not surprising that disruptions in GABAergic circuits are leading risk factors for many cognitive disorders. The atlas and methods we are generating will thus also serve as a reference data set and standard technology for determining the GABAergic circuit defects in mouse models of human disorders, including epilepsy, schizophrenia, autism, depression, and attention deficits.

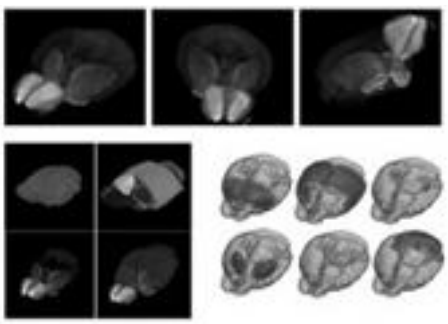

Figure 2. Reconstruction of GABAergic neurons in the mouse brain by STP tomography, using GAD-Cre knock-in mice generated by Josh Huang's lab at CSHL. (*Top* panels) Imaged GABAergic neurons, (*bottom* panels) anatomical segmentation into specific brain regions.

Summary

We hope that our work will lead to a better understanding of how neural circuits generate complex behaviors and how such circuits are disrupted in autism and schizophrenia.

PUBLICATIONS

In Press

Glajch KE, Fleming SM, Surmeier DJ, Osten P. 2012. Sensorimotor assessment of the unilateral 6-hydroxydopamine mouse model of Parkinson's disease. *Behav Brain Res* **230:** 309–316.

Ragan T, Kadiri LR, Venkataraju KU, Bahlmann K, Sutin J, Taranda J, Arganda-Carreras I, Kim Y, Seung HS, Osten P. 2012. Serial two-photon tomography for automated ex vivo mouse brain imaging. *Nat Methods* **9:** 255–258.

NEURAL CIRCUITRY FOR SOCIAL COMMUNICATION

S. Shea H. Demmer G. Ewall
D. Eckmeier M. Manglani

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 on 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 the autism spectrum disorders (ASDs); 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.

There are three broad areas of research in the lab. One concerns olfactory communication and memories for familiar individuals. Another direction is an examination of vocal (auditory) communication between mother mice and their pups. Our final avenue of study involves 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

H. Demmer, D. Eckmeier, G. Ewall

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 the locus coeruleus (LC), 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 a variety of 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 plasticity 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.

How are olfactory memories for individuals stored mechanistically among the specific synaptic connections of the various neuronal types in the olfactory bulb? To answer this question, one of our group, Heike Demmer, developed techniques for making technically challenging targeted recordings from a specific type of inhibitory neuron (granule cells) whose function remains mysterious. She then made these recordings during the induction of NA-dependent plasticity to examine how they contribute to memories. The data suggest that these cells not only participate in storing olfactory memories, but do so in surprising and complex ways. Although we anticipated that the granule cells might increase their activity to suppress responses to remembered odors, we found the opposite was true. One interpretation is that NA input uncouples granule cells' synaptic output from their action potential firing. This unique property could be an important factor in maintaining memory specificity.

This year, in addition to solidifying the above findings, Heike has begun making even more challenging and sensitive recordings from granule and mitral cells. The data provide the first compelling evidence that naturalistic stimulation of NA release in vivo suppresses granule cells and alters the temporal pattern of their firing (Fig. 1), thus relieving mitral cells from their inhibition. We believe these findings are important clues to the fundamental mechanisms by which NA stores memories in the olfactory bulb. Disinhibition of mitral cells may be a key trigger for the synaptic plasticity that likely underlies memory.

We have been testing this model using cutting-edge optogenetics techniques to activate and inactivate isolated circuit elements with light in order to establish their causal role in the general phenomenon. We have shown that the basic features of NA-dependent plasticity can be recapitulated using light-mediated neural stimulation to replace sensory stimulation. To follow up, we have shown that by isolating different parts of the olfactory bulb circuit, we can constrain the key neural activities that alter behavior. For example, we are planning to use disinhibition of mitral cells by optogenetics techniques to recreate the neural dynamics that we now know are set up by NA and assess its sufficiency for plastic changes.

Dennis Eckmeier has been taking a different approach to observe NA-dependent memory formation with functional neural imaging. Figure 2A shows a sample odor response image taken from the left and right olfactory bulbs of a mouse. The dark spots show parts of the brain that are activated by the odor. By plotting

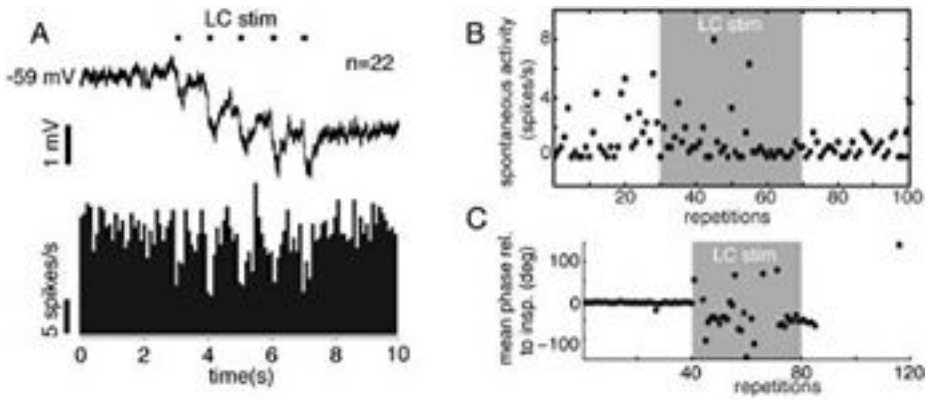

Figure 1. LC stimulation acutely suppresses glucocorticoids (GCs). (*A, Top*) Average median filtered (5 ms) trace taken from 22 stim trials of a GC recorded in the "whole cell" configuration. Stimuli were five 20-Hz bursts of five pulses delivered at 1 Hz to LC. (*A, bottom*) PSTH (poststimulus time histogram) of spiking taken from the same trials. Bins = 100 ms. (*B*) LC stimulation acutely suppresses spontaneous firing in many GCs. Mean spontaneous firing rate in each 30-s trial is plotted for 100 trials. LC stim during the shaded period is a 5-s, 5-Hz, 50-μA train once per trial. (*C*) LC stimulation acutely disrupts the phase of firing relative to inspiration in many GCs. Mean phase angle of spiking, calculated as the angle of the vector sum of all spikes recorded on each 30-s trial, is plotted for 120 trials. Inspiration = 0°. LC stimulation during the shaded period is a 5-s, 5-Hz, 50-μA train once per trial. Trials that are missing a datapoint were trials that did not exhibit enough spiking activity to accurately measure phase.

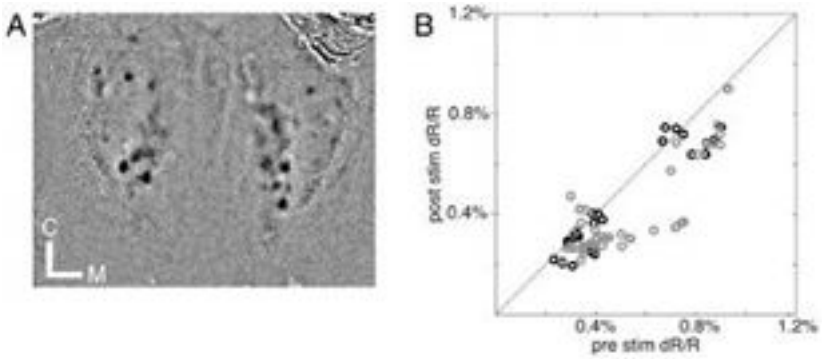

Figure 2. LC stimulation suppresses population responses to paired odors. (*A*) A typical ISI (intersymbol interference) response to cineole in the MOB (main olfactory bulb). Dark spots indicate active glomeruli. (*B*) Scatterplot of the response of 83 glomeruli from seven mice, plotting post-LC stimulation response strength over pre-LC stimulation response strength. (*Black circles*) From the contralateral bulb, (*gray circles*) from the ipsilateral bulb.

the response strength of each of these spots before and after NA release, Dennis has shown that NA weakens the response to paired odors. This effect is only observed in the stimulated bulb (Fig. 2B). These preliminary data open the door to observing the encoding of memories at the neural population level.

How are changes in the firing rate of olfactory bulb neurons read out by deeper stations in the brain? It has been hypothesized that NA may modulate behavior by suppressing input to downstream targets that mediate innate behavioral responses. To begin testing this hypothesis, we have made recordings from some of these deep-brain structures, including the bed nucleus of the stria terminalis and the medial amygdala. We were able to demonstrate that these regions respond robustly to biologically important stimuli such as urine from the opposite sex and predator odors. This work sets the stage for examining how NA shapes these responses and relating these effects to different behaviors.

Vocal Communication between Mothers and Pups

M. Manglani

Far outside the range of our hearing, in the ultrasound range, 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 ultrasonic distress vocalization (USV). Young mice prior to vision and full mobility 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. Moreover, nulliparous females who have never given birth do not show approach responses to pups or their calls, suggesting that the underlying neural responses may also differ.

Evidence suggests that vocal selectivity and the changes in behavior between mothers and nulliparous females may be related to inhibition in the auditory cortex. We are using optogenetic inactivation of inhibitory cortical interneurons to test this hypothesis. Our summer URP Monica Manglani began these experiments this year and was able to show that our viral strategy for expression of optogenetic tools is effective and allows us to disinhibit cortical neurons, thus affecting their selectivity (Fig. 3).

Neural Activity during Social Encounters

D. Eckmeier

We have begun experiments that are ultimately aimed at recording individual neurons during social encounters and other behavioral assays involving the perception

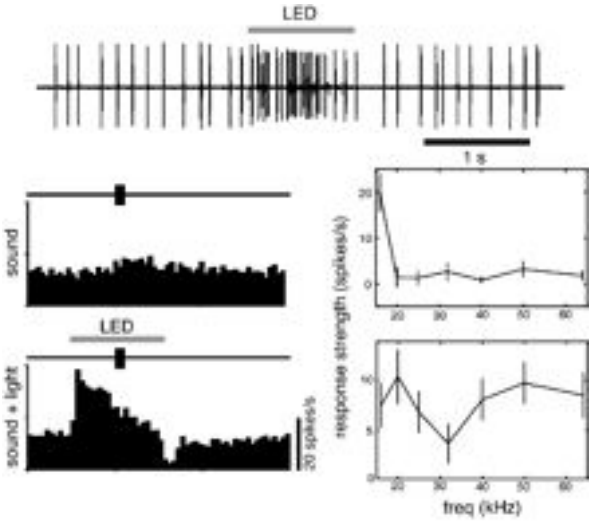

Figure 3. Inhibition shapes auditory cortical tuning. (*Top panel*) Response of an auditory cortical neuron in a mouse in which auditory cortical interneurons are expressing an optogenetic tool that allows them to be inactivated by light. Depicted beneath are peristimulus time histograms and tuning curves showing how light inactivation of inhibitory neurons affects the mean response and frequency tuning.

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.

Critical to this approach is the development of techniques for observing neural activity with implantable probes that are sufficiently lightweight and low profile to not interfere with normal social behaviors, yet are also stable enough to allow steady, low-noise recordings during highly interactive behaviors such as mating. Our initial experiments make use of fixed silicon probes etched with a high-density array of electrical contacts. The data show that these probes can be used to record multiple isolated neurons in the socially significant modulatory nucleus locus coeruleus. We are now using these probes in awake animals to assess the structure of LC output during social learning with high temporal resolution.

PUBLICATION

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Heike Demmer and Steve Shea

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 different memories. These memories are, under normal circumstances, highly accurate for particular stimuli and situations. This is precisely the facility that is lost in diseases such as Alzheimer's and other dementias. Our overall goal is to understand how the brain forms such precise memories: How does the specificity arise, and how does the brain derive such tremendous capacity for forming different memories?

We address these questions by studying olfactory memory formation in *Drosophila*. Just like Pavlov's dogs, *Drosophila* learn to form associations between smells and reward or punishment. A specific area of the fly brain, known as the mushroom body (MB), is essential for the flies to form olfactory memories. We are investigating how the neural activity patterns in this brain area are used to form specific olfactory memories. To achieve this, we monitor activity using both electrophysiological and functional imaging techniques. We have found that MB neurons exhibit highly odor-specific responses and that activity patterns are relatively sparse across the population of MB neurons. This specificity is thought to underlie the accuracy of memory because modifying the synapses of highly odor-specific neurons would lead to relatively precise memories. Sparse representations by highly stimulus-specific neurons are a general feature of brain areas involved in learning and memory, including hippocampus and cerebellum.

Using the simplicity and genetic manipulability of *Drosophila*, our goal is to understand several fundamental properties of neural responses in learning- and memory-related brain areas: What mechanisms give rise to the stimulus specificity? What exactly is meaningful about the activity patterns: Is it simply which cells respond or does the precise timing of activity matter? How are these response patterns modified by learning? And ultimately, what is the role of the many genes implicated in learning with respect to network-level activity in the brain?

Robust Sparse Coding in the MB

K. Honegger, R. Campbell

Theoretical studies indicate that sparse representations are useful for learning and memory. However, it is crucial to distinguish between truly sparse representations and simply weak responses observed because the stimuli tested are suboptimal (i.e., not those the system evolved to detect) or because strongly responsive cells are spatially segregated. In this study, we used imaging to establish that odor representations are genuinely sparse in the MB. We showed that natural stimuli that have intrinsic meaning to the fly do not elicit qualitatively distinct responses from artificial, monomolecular odors. Additionally, there is no evident spatial segregation of responses which would suggest that olfactory information is channeled into distinct anatomical pathways. Rather, odors evoke responses in a small fraction of the MB population, with cells randomly distributed throughout the MB.

This format is likely to be useful for memory storage and recall. It is a simple way to minimize the overlap between representations of different stimuli: If each odor evokes a response in 10% of the MB neurons, and for each odor, the 10% are randomly distributed across the population, the representation of any two odors will overlap by only 1%. Consequently, any changes in the properties of neurons that respond to one of the odors should only have a tiny effect on the representations of other odors. Overall, this format optimizes the circuit to form accurate memories with specific odors. We examined this explicitly in the next project.

MB Population Responses Predict the Specificity of Olfactory Learning

R. Campbell, K. Honegger, E. Demir, H. Qin, W. Li

Do the neuronal response patterns in the MB convey the specificity of memory formation? If so, what fea-

tures of neural activity underlie that specificity? To address these fundamental questions, we tested whether we could use the activity patterns we measure in the MB to predict the odor specificity of flies' memories. We examined this in two contexts: when flies learn (1) fine discriminations between highly similar odors and (2) an association with one odor and then they generalize that association to a different but related odor.

To assess the odor specificity of the memories, we used the standard Pavlovian conditioning protocol, training flies to form an association with one odor and testing the specificity of those memories by giving flies a choice between that odor and a second, similar but distinct, odor. We compared this behavioral measure with the specificity of the response pattern of ~150 MB neurons to these odors. We found that the MB activity patterns accurately predict the specificity of memory formation. This prediction held true even as we approached the limit of how accurately flies learn.

To evaluate the features of neural activity that underlie accurate memory formation, we developed techniques to track activity of ~80% of the 2000 neurons in the MB. The unprecedented scope of these experiments enabled us to find the small differences between different odor representations. We found that, even when flies are forced to choose between two odors that are near the limit of their discrimination ability, there are neurons that respond to only one odor of the pair, so that binary differences are present between the two odor representations. These differences are sufficient to train a biologically realistic model network to respond specifically to different odors. Two important conclusions from this study are that (1) although response patterns in the olfactory receptor neurons (ORNs) differ in the relative levels of activity across different ORNs (analog coding), these differences are converted into simple on/off (binary) differences in response patterns at the MB and (2) a simple learning scheme of changing synaptic strength of all neurons that respond to an odor is sufficient to accurately determine odor identity.

These results show how a simple coding scheme can enable accurate but generalizable memory formation. They also suggest that it may be possible to restore memories with coarse interventions that simply drive neurons to respond and that precise control of spike timing may not be needed.

Integrating the Olfactory Combinatorial Code at the Dendrites of MB Neurons

E. Gruntman

How is it possible to recognize an odor as a unified smell (e.g., coffee) when it is composed of so many different components? One idea is that ORNs in the nose (fly's antennae) each recognize a particular feature of an odor, and the overall combination of activated ORNs conveys the identity of the odor. For this combinatorial coding scheme to work, there must be neurons at deeper layers of the brain that respond to specific combinations of coactive inputs. In this work, we show it is the MB neurons that read the combinatorial code of the earlier layers of the system.

We addressed this issue by examining odor responses of individual dendritic input sites on the MB neurons, making this one of only a handful of studies to examine sensory responses of dendrites in vivo. To achieve this, we expressed a calcium-sensitive fluorescent protein in single MB neurons and then used two-photon imaging to examine the responses of each dendritic site to a panel of different odors. Using this approach, we could construct an odor-tuning curve for each dendritic input site. By comparing tuning curves for different dendritic sites, we directly showed that some MB neurons integrate combinations of different inputs. We also find evidence that, for a small fraction of MB neurons, all of the inputs are identical. These neurons may act as amplifiers, summing up weak but identical signals from multiple synaptic contact sites.

We have also devised an approach to test whether multiple different inputs must be active in order to drive the MB neuron to spike. Our early results here suggest an elegant explanation for why MB neurons are so odor-selective: They not only *receive* different types of inputs, but they *require* those different inputs to be coactive in order for the MB neuron to spike.

Decoding Sparse Representations

T. Hige

Sparse representations are useful for learning and memory, but how do downstream neurons integrate this information? Ultimately, the information in layers with sparse representations must be converted into a behavioral response. How does this process occur, and how

do neurons downstream from a sparsely responding brain area integrate that information?

In *Drosophila*, this process appears to occur immediately after the MB, as the 2000 MB neurons converge onto an estimated 75 output neurons. Individual members of this output population can be labeled with green fluorescent protein (GFP) using the genetic tools available in *Drosophila*, making this an excellent system to determine the basic principles of information processing downstream from sparse representations. Using GFP-targeted intracellular recordings, we have characterized the odor responses of one of these MB output neurons. In contrast to the highly odor-specific tuning of MB neurons, this output cell responds to a broad array of different odors. These functional properties are reflected in the cell's anatomy: It has very extensive dendritic processes within the output lobes of the MB, suggesting that it receives highly convergent input from many MB neurons. Although the neuron responds to almost all tested odors, it nevertheless responds distinctly to different odors, suggesting that this neuron is capable of carrying odor-specific information. However, the odor-tuning properties of this neuron vary between individual flies; the same neuron has very different tuning curve shapes in different animals. One explanation for this variability is that it reflects synaptic plasticity operating on a developmental timescale. Our working hypothesis is that the tuning curves of the MB output neurons are a product of the prior olfactory experience of the individual fly. We are currently testing this by examining the role of olfactory experience in shaping the response properties of these neurons.

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Robert Campbell, Glenn Turner, Kyle Honegger, Eyal Grutman, and Toshi Hige

NEURAL CIRCUITRY OF UNDERLYING NORMAL AND ABNORMAL PROCESSING IN THE CORTEX

A. Zador K. Borges, B. Burbach, G. Cao, T. Hromadka, S. Koh, H. Oviedo, H. Oyibo, S. Peikon, A. Reid, Q. Xiong, H. Zhan, 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.

Sequencing the Connectome

G. Cao, H. Oyibo, S. Peikon, H. Zhan [in collaboration with J. Dubnau and L. Enquist, Cold Spring Harbor Laboratory]

The brain is a complex network, consisting of billions of neurons connected by trillions of synapses. The details of these connections—which neurons form synaptic connections with which other neurons—are crucial in determining brain function. Malformation of connections during prenatal and early postnatal development can lead to mental retardation, autism, or schizophrenia; loss of specific connections later in life is associated with neurodegenerative diseases such as Alzheimer's. An efficient method for determining the brain's wiring diagram would transform neuroscience research.

Inspired in part by the success of the Allen Brain Atlas, the human genome project and other major efforts, there is growing excitement in neuroscience to determine the complete connectivity diagram—the "connectome"—of the brain. So far, the complete connectome has been established for only one organism, the tiny worm *Caenorhabditis elegans,* with 302 neurons connected by ~7000 synapses. However, determining the connectome of even this simple nervous system was a heroic task, requiring more than 50 person-years of labor to collect and analyze the electron micrographs.

All current approaches to determining the connectome are based on microscopy. Unfortunately, microscopy is poorly suited to the study of neural connectivity because brains are macroscopic structures, whereas the synaptic connections between neurons require electron microscopy. Reconstructing the complete wiring diagram of such a brain is akin to piecing together the complete roadmap of the United States from a collection of postage-stamp-sized photographs.

To circumvent the considerable challenges associated with determining the connectome based on microscopy, we are developing an entirely novel approach based on high-throughput DNA sequencing technology. Sequencing technology has not previously been applied in the context of neural connectivity. The appeal of using sequencing is that it is already fast—sequencing hundreds of millions of individual pieces of DNA in a single day is now routine—and, like microprocessor technology, getting faster exponentially. Moreover, the cost of sequencing is plummeting: Predictions are that it will be possible to sequence an entire human genome (~3B nucleotides) for $1000 within a few years. Thus, by converting brain connectivity from a problem of microscopy to a problem of sequencing, we render it tractable using current techniques.

An inexpensive and rapid method for deciphering the wiring diagram of a neural circuit of an entire brain would have a profound impact on neuroscience research. Knowing the neuronal wiring diagram would provide a foundation for understanding neuronal function and development, in the same way that knowing the complete genomic sequence provides the starting point for much of modern biological research in the postgenomic era. Moreover, many neuropsychiatric diseases such as autism and schizophrenia are thought to result from disrupted neuronal connectivity, but identifying the disruptions even in mouse models is a major challenge given current technology. Our approach may help to usher in an era when understanding the connectivity of a new mouse model of neuropsychiatric disease is the routine first step to analyzing its deficits.

Disruption of Auditory Cortical Circuits by Autism Candidate Genes

Q. Xiong, H. Oviedo

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. We recently found that the autism candidate gene *PTEN* disrupts local and long-range connections (Xiong et al. 2012).

Role of Interneurons in Auditory Cortex Function

A. Reid, T. Hromadka

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

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

Processing of Spatial Information in the Auditory Cortex

S. Koh

In vision and somatosensation, spatial information is already present at the receptor level. In audition, spatial information is not available at the cochlear level and must be computed using information from both hemispheres. A subpopulation of neurons in the primary auditory cortex conveys sound location and sound motion information, but little is known at the neural circuit level because of technical difficulties of identifying neurons during in vivo recordings.

We have previously developed a technique called PINP (photostimulation-assisted identification of neuronal populations) which allows us to "tag" subpopulations of neurons based on their axonal projections. The tag is a light-gated ion channel, channelrhodopsin-2 (ChR2), that can be triggered by a brief flash of blue laser with millisecond precision. We inject herpes simplex virus (HSV), which can be taken up by axons and travels in a retrograde fashion, in order to deliver ChR2 specifically to the neurons projecting to the infected area. ChR2-tagged neurons can be identified during in vivo recording by responsiveness to a flash of blue light.

We are using PINP to test the hypothesis that layer-3 ACx neurons projecting to the contralateral ACx are more sensitive to auditory spatial information than other subpopulations in ACx. Layer-3 neurons seem to be ideal candidates, deduced from the facts that (1) many layer-3 ACx cells project to contralateral ACx, unlike visual and somatosensory cortex where layer-3 cells predominantly project to ipsilateral cortical areas, and (2) processing of spatial information requires inputs from both hemispheres.

Circuits Underlying Auditory Representations and Decisions

S. Jaramillo, P. Znamenskiy, K. Borges

We are studying the circuits underlying auditory representations and how these representations give rise to decisions. We train rats and mice to perform well-controlled auditory tasks and then use electrical and optogenetic methods to monitor and perturb neural activity. Using this approach, we recently found that the auditory cortex mediates the perceptual effects of acoustic temporal expectation (Jaramillo and Zador 2011). We are now extending this approach to study the role of the auditory thalamus, cortex, and striatum in these behavioral processes.

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong J. Beshel A. Sodsiri
Y. Shuai C. Xu

Olfactory Trace Conditioning in the Fruit Fly

Y. Shuai

Trace conditioning is a simple experimental model to assess how the brain associates events that are discrete in time. To study trace conditioning in the fruit fly, we trained the animals to avoid an odor that is followed by foot shock many seconds later. The molecular mechanisms of this form of learning are surprisingly distinct from the well-characterized simultaneous conditioning, where odor and punishment temporally overlap. First, rutabaga adenylylcyclase (Rut-AC), a putative molecular coincidence detector vital for simultaneous conditioning, is dispensable in trace conditioning. Second, dominant-negative Rac expression, thought to sustain early labile memory, significantly enhances learning of trace conditioning, but it leaves simultaneous conditioning unaffected. Targeting Rac inhibition to the mushroom body (MB) suffices to achieve the enhancement effect, which suggests the MB as a crucial neuroanatomical locus for trace conditioning. We show in a working model that the MB may harbor a Rac activity-sensitive olfactory "sensory buffer," which bridges the temporal gap between odor and foot shock in trace conditioning. Live animal imaging is now being applied to characterize the nature of this putative sensory memory.

Value Coding in the *Drosophila* Brain

J. Beshel

Odors are highly evocative, yet how and where in the brain odors derive meaning remains unknown. We first established *Drosophila*'s partiality for differing food odors and then showed that odor-evoked activity of neurons located outside of the canonical olfactory system precisely determines odor value. In vivo two-photon calcium imaging of the fly brain revealed that the responsiveness of *Drosophila* neuropeptide F (dNPF), the neuropeptide Y homolog, was much greater to food odors than to nonfood odors, including food-odor components. Moreover, hunger similarly elevates neural and behavioral food-odor responses. Remarkably, these neurons are not only necessary, sufficient, and specific to food-odor attraction, but the amount of activity exactly defines attraction level to, and even preference between, individual food odors. We thus demonstrated the existence of a motivationally scaled neural "value signal" accessible from uniquely identifiable cells. Responses are graded within a specific class of stimuli and stable across individuals, making precise predictions about behavior possible.

NF1 and Memory in *Drosophila*

C. Xu

The ras/mek/MAPK (mitogen-activated protein kinase) pathway has been implicated as an important pathway for cognitive function by linkage mapping studies. Neurofibromatosis type 1 (NF1) is a single-gene disorder caused by mutations in the gene neurofibromin (*NF1*). NF1 encodes the GAP domain and thus functions as a negative regulator of the ras pathway. *Drosophila melanogaster* has an *NF1* gene that shares high similarity with the human *NF1* gene. *NF1*-null mutant flies have both short- and long-term memory defects, and the defects can be rescued by reexpressing the *NF1* gene construct specifically in the MB—the memory center for flies. Overexpression of dominant-negative ras postdevelopmentally in the MB also severely affects long-term memory, suggesting that the manner in which NF1 has been involved in the long-term memory process is ras-pathway dependent. MAPK has been shown to have a critical role in LTP (long-term potentiation) formation and memory processes. *NF1*-null mutants have abnormally high MAPK activity, which suggests that the long-term memory defects in *NF1* mutant flies is probably caused by MAPK disregulation. We are currently investigating how MAPK is regulated during memory processes and the role of *NF1* in the long-term memory process.

Drosophila as a Model to Study Nonsyndromic Mental Retardation

A. Sodsiri

ARHGEF6 is one of the genes that have been linked to nonsyndromic mental retardation. It encodes Rho-type guanine nucleotide exchange factor (Rho-GEF), which can switch on Rho GTPases by mediating the exchange of GDP for GTP. A *Drosophila* homolog of this gene, dPix, has been shown to regulate postsynaptic structure and protein localization at the *Drosophila* neuromuscular junction. We set out to study the role of Rho-GEF in learning and memory in *Drosophila* and to characterize the regions in the fly brain whereby dPix functions. In our preliminary study, we found that mutation in dPix or knocking down of dPix by RNA interference (RNAi) produces learning deficits in *Drosophila*. Moreover, knocking down of dPix in the MB throughout development causes the fly to have a significantly smaller MB, which might explain the observed learning deficit because the MB is the central site for associative learning. Investigation into these behavioral and anatomical phenotypes will give insight into mechanisms underlying learning disabilities seen in patients with nonsyndromic mental retardation.

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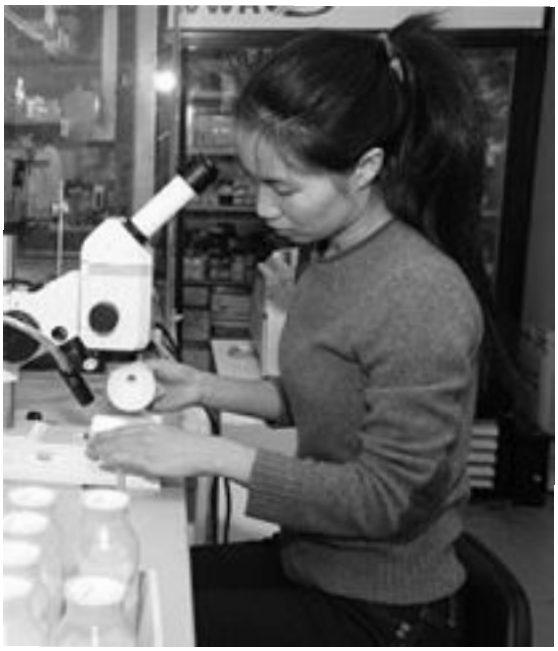

Chunsu Xu

PLANT GENETICS

Uncovering the molecular processes governing how plants grow and develop is of fundamental biological interest and importance, with vital implications both for agriculture and human health. *Plant genetics* has had a major role in the advance of all areas of research at CSHL almost since the Laboratory's inception and is epitomized by the work of Nobel laureate Barbara McClintock, who discovered transposable genetic elements in her work on maize in the 1940s. Plant geneticists at CSHL 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 wide range of cereal crops that feed the planet's growing population. CSHL plant geneticists also have been pioneers in the study of RNA interference, stem cell research, and, most recently, 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. They are investigating a unique way in which plant cells communicate, by transporting regulatory proteins via small channels called plasmodesmata. These channels, which direct the flow of nutrients and signals through growing tissues, are regulated during development. The team discovered a chaperone protein-encoding gene, *CCT8*, that controls the transport of a transcription factor, KNOTTED1, between cells in the plant stem cell niche, or meristem. KNOTTED1 is critical for stem cell maintenance, and studies of the *CCT8* gene indicate that movement of KNOTTED1 between cells is required for this function. The lab also continues to identify other genes that control plant architecture through effects on stem cell maintenance and identity. Recent examples include discovery of a subunit of a heterotrimeric G protein that is conserved throughout animals and plants, and these studies indicate that this gene controls stem cell proliferation. A second example is the identification of the gene *GRASSY TILLERS1* that appears to have been instrumental in the domestication of the corn plant, a process that led to the development of agriculture almost 10,000 years ago. This past year, the Jackson team demonstrated that *gt1* expression is associated with the suppression of tiller growth and amassed evidence indicating that *gt1* was specifically (if unwittingly) selected by ancient agriculturalists in their attempts to domesticate teosinte. Separately, the lab has characterized system-wide networks of gene expression in inflorescence development, using "next-gen" profiling methods, and is developing a collection of maize lines that can drive expression of any reporter or experimental gene in any tissue type. These tools are of great interest to maize researchers and are being made available to the broader scientific community, enabling experiments never before possible in crop plants.

Zach Lippman's research focuses on identifying genes that cause tomato plants to produce their flowers in an elegant repetitive zigzag arrangement on a branching structure called an "inflorescence." Proceeding from a base of knowledge that they have built regarding a universal growth habit represented by tomato, called sympodial growth, Lippman's lab is addressing the question of zigzag flower patterning by focusing on the fundamental question of how plants are able to stop making leaves and start making flowers. Of particular interest is how these "reproductive phase transitions" have contributed to the evolution of diverse inflorescence branching patterns in the tomato's larger Solanaceae family, which includes plants that make just one flower in each inflorescence, to plants whose inflorescences produce dozens of branches and hundreds of flowers. Using classical and modern genetic techniques, Lippman is dissecting the gene networks that are responsible for the variation in inflorescence branching found in nature. He hopes to leverage these discoveries to improve crop yields in several solanaceous plants such as pepper, eggplant, and potato. Already, a collaboration between Lippman's group and scientists at Hebrew University has identified a gene called *florigen* that is responsible for making flowers in plants and can be altered using simple genetic tricks to push hybrid tomato plants to increase their yield by as much as 60%. This past year, the lab used a

systems biology approach and next-generation sequencing technology to capture the transcriptome of stem cells at five different stages of maturation, which enabled the team to identify nearly 4000 genes that represent the "maturation clock" in tomato plants.

Epigenetic mechanisms of gene regulation—chemical and conformational changes to DNA and the chromatin that bundles it—have important impacts on genome organization and inheritance as well as on cell fate. These mechanisms are conserved in eukaryotes and provide an additional layer of information superimposed on the genetic code. Robert Martienssen, a pioneer in the study of epigenetics, investigates mechanisms involved in gene regulation and stem cell fate in yeast and model plants including *Arabidopsis* and maize. He and colleagues have shed light on a phenomenon called position-effect variegation, 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. Recently, 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 transposon damage. With collaborators in Mexico, Martienssen has also coaxed *Arabidopsis*, a flowering plant, to produce egg cells without meiosis, an important step toward a long-time goal of plant breeding: generating clonal offspring to perpetuate hybrid vigor. This past year, the lab published research revealing that when RNA polymerase II has transcribed a stretch of DNA, the RNAi mechanism causes the enzyme to release its hold on the DNA and fall away. This allows the replication fork to progress smoothly and the DNA strands to be copied; histone-modifying proteins, which follow right along, establish heterochromatin. 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 that act as morphogen-like signals. The lab's most recent findings identified a third small RNA gradient involved in maintenance of organ polarity, illustrating the complexity with which small RNAs generate developmental patterns. Mathematical modeling suggests that such opposing small RNA gradients might serve to generate robustness during development.

DEVELOPMENTAL BIOLOGY: STEM CELLS, SIGNALING, AND CONTROL OF PLANT ARCHITECTURE

D. Jackson K. Ackerley, P. Bommert, C. Brooks, S. DeBlasio, M. Elouet, A. Eveland, V. Galvan, S. Goldschmidt, B.I. Je, A. Kolbe, L. Marshall, R.T. Morales, M. Pautler, H. Szilagyi, Y.G. Wolfenson, X.M. Xu, F. Yang, T. Zadrozny

Our research aims to identify genes, signals, and pathways that regulate plant 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 are just beginning to emerge. Plasmodesmata are critical for plant growth because they allow the passage of nutrients and signals through growing tissues. We have elucidated an important mechanism by which transport through these channels is controlled via a chaperonin gene that regulates the specific transport of a transcription factor, KNOTTED1 (KN1). This factor is a homeodomain protein that is expressed in the plant stem cells and is necessary to keep these cells in a pluripotent state. We found that KN1 function depends on chaperonin activity, highlighting the importance of cell-to-cell trafficking in plant stem cell function.

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* (*CT2*), encodes a subunit of a heterotrimeric G protein that has previously not been linked with stem cell proliferation in plants. Studies of *CT2* have identified a novel signaling pathway that controls stem cell proliferation. Other newly identified genes include those controlling maize branching in response to light signals and maize ear size. 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.

Characterization of a Dominant Phyllotaxy Mutant, *Abphyl2*, in Maize

F. Yang, A. Kolbe, D. Jackson [in collaboration with D.V. Llaca and H. Sakai, DuPont Crop Genetics, Wilmington, Delaware]

Plant morphology and diversity are largely dependent on phyllotaxy, which is the geometric arrangement of new primordia formed by the shoot apical meristem (SAM). Biologists and mathematicians have wondered for centuries how these patterns, such as the beautiful sunflower head spirals, are generated. Recent studies found that a plant hormone, auxin, and its polar transport by PIN-FORMED1 (PIN1) proteins, is crucial for controlling phyllotactic patterns. Recently, our studies on *ABPHYL1* in maize have shown that cytokinin hormone signaling, as well as its cross-talk with auxin, have an important role in this process. *abphyl1* (*abph1*) mutations change maize phyllotaxy from alternate to decussate. Here, we describe a similar phyllotaxy mutant, *Abph2*. *Abph2* is dominant, and it originated from a Chinese inbred line and has a decussate leaf pattern that becomes visible at the four-to-five leaf stage. Map-based cloning located the *Abph2* locus to a region of ~ 20 kb on chromosome 7, containing five predicted genes in the reference B73 genome. However, sequencing of the open reading frames (ORFs) of these five genes, as well as their transcript level analysis by RT-PCR (reverse transcriptase–polymerase chain reaction), did not give any obvious clues as to the identity of the *Abph2* gene. Given the dynamic nature of the maize genome, we hypothesized that a gene not present in the reference B73 genome could be responsible for the *Abph2* phenotype. We therefore made a bacterial artificial chromosome (BAC) library from the *Abph2* mutant and screened it using probes located within the 20-kb

mapping interval. We identified a positive clone that covered the entire interval, and BAC sequencing revealed a 4.5-kb fragment inserted into the mapping interval relative to the B73 reference genome. This inserted fragment contained a predicted *glutaredoxin* (*GRX*) gene that was identical to a gene located ~800 kb distal in the B73 genome. To determine whether the transposed 4.5-kb fragment was responsible for the *Abph2* phenotype, we made maize transgenic lines containing this 4.5-kb region with yellow fluorescent protein (YFP) fused to the *GRX*-coding sequence. These transgenic lines phenocopied the *Abph2* phyllotaxy phenotypes, demonstrating that this *GRX* gene is the cause of the decussate leaf phenotype. Meanwhile, knockout lines of the dominant *Abph2* mutation were generated by EMS (ethylmethanesulfonate) mutagenesis. Sequencing the *GRX* gene in these knockout lines found point mutations leading to conserved amino acid changes in two independent lines, further supporting the idea that the transposed *GRX* gene is responsible for the *Abph2* phenotype.

RNA in situ hybridization was used to localize *Abph2* transcripts, which accumulate in the P zero (P_0) leaf initiation site and vasculature in vegetative meristems (Fig. 1). More careful investigation showed that *Abph2* is expressed first in the leaf vasculature at 20 days after pollination, and later on, it becomes localized to the P_0. Further analysis will focus on how this transposed *GRX* gene changes the leaf pattern as well as the meristem size.

Finally, informed by the fact that auxin patterning and PIN1 maxima in the leaf initiation site are diminished in *abph1*, we are currently analyzing auxin distribution in *Abph2* meristems, using PIN1-YFP and DR5-RFP (red fluorescent protein) as markers. Preliminary analysis revealed that the expression of PIN1 in the *Abph2* SAM appears to be decreased, suggesting a common mechanism by which these mutants control phyllotaxy.

Regulation of the Cell-to-Cell Trafficking of the Transcription Factor KNOTTED1

X.M. Xu, R.T. Morales, V. Galvan

Cell-to-cell communication functions in specifying cell fate and coordinating development in all multicellular organisms. A special paradigm for such communication in plants is the selective trafficking of signaling macromolecules, e.g., transcription factors and small RNAs through plasmodesmata (PDs), channels that traverse the cell wall and connect all plant cells. In addition to cell-fate specification, PDs are also involved in viral movement, transport of metabolites, and cell-to-cell spread of RNA interference (RNAi), which points to their fundamental importance in coordinating plant defense, metabolism, and development. Despite the discovery of PD more than 100 years ago, and our increasing recognition of their functional significance, the underlying components and mechanisms of PD trafficking remain poorly understood. Hence, we are taking an unbiased genetic strategy to dissect these molecular components and mechanisms, using a transgenic reporter system. We used this system to identify mutants that affect KN1 transport. One mutant that we have identified encodes a chaperonin. Genetic interaction data provide evidence that the endogenous function of KN1-related genes in *Arabidopsis* requires chaperonin, clearly supporting the functional relevance of chaperonin-mediated trafficking through PD (Fig. 2). Our results suggest that chaperonin dependency is a general mechanism for protein trafficking and highlight the importance of conformational changes for PD trafficking. Current efforts are focused on isolation of additional mutants and their identification by next-generation sequencing.

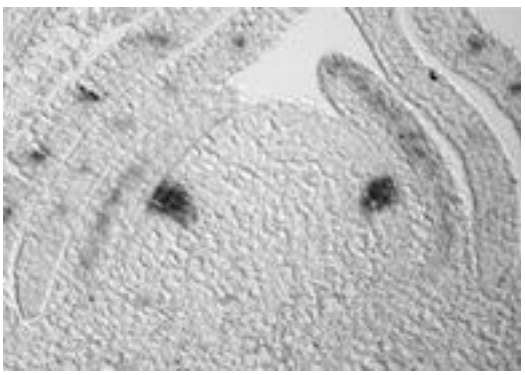

Figure 1. Highly localized expression of *ABPHYL2* in "P zero" domains of the shoot apical meristem.

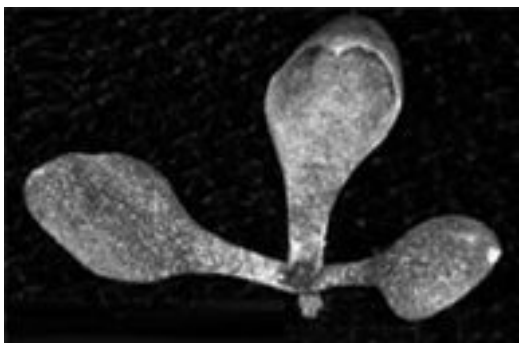

Figure 2. An *Arabidopsis* double mutant, between a weak *shoot-meristemless* allele and the *cct8* mutant, shows enhanced stem cell defects.

Illuminating Maize Biology: Using Fluorescent Proteins for High-Throughput Analysis of Protein Localization and Function in Maize

S. DeBlasio, A. Goldschmidt, T. Zadrozny, J. Wang
[in collaboration with A. Chan, J. Craig Venter Institute;
A. Sylvester, University of Wyoming]

Recent advances in sequencing technology have generated thousands of publicly available genome sequences from nearly all groups of organisms. These massive data sets have revolutionized the types of biological questions that can now be addressed. A continued challenge is to ascribe function to the high percentages of predicted proteins and proteins of unknown function. Despite the ability to predict the function of some proteins by comparative bioinformatics, experimental validation remains necessary to assign function definitively. One of the most effective approaches to validate gene function is to develop visualization and biochemical tools associated with fluorescent protein (FP) technology.

When exposed to light, FPs fused to a protein or regulatory element fluoresce, making the attached normally invisible protein visible and easy to track under a microscope. 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, in vivo in real time. We have successfully optimized this technology for maize and have created a public resource of cellular biomarkers that we and other members of the plant community use both for small-scale analysis of individual genes and for global expression and functional profiling. To date, we have generated more than 100 stable, natively expressed, maize FP fusion lines that highlight most subcellular compartments and allow us to study various cellular pathways including hormone signaling, cytoskeletal behavior, vesicle trafficking, and stem cell maintenance and differentiation. We are also using these lines to immunoprecipitate tagged proteins to identify previously uncharacterized components of these important pathways. In the case of FP-tagged transcriptional regulators, chromatin immunoprecipitation (ChIP) is being used to identify putative DNA-binding sites and/or to confirm gene targets that have been detected by transcriptional profiling of the respective mutant.

To study maize development on a broader scale, we are optimizing the LhG4 two-component *trans*-activation expression system for use in maize. This system relies on the transcription factor LhG4 being expressed by a cell or tissue-specific promoter to drive the expression of a reporter in *trans*, via the pOp-binding sites. The reporter can be any gene, such as GUS, FPs, an FP fusion protein, a gene of interest for tissue-specific expression, a silencing construct for transcriptional knockdowns, or a gene encoding a toxin for tissue ablation experiments. We have already generated an overexpression promoter construct and are currently working to create several meristem- and leaf-specific LhG4 drivers, including p*RAMOSA3,* p*WUSCHEL*, and p*YABBY14*. Our goal is to produce an array of cell- and tissue-specific LhG4 lines, which we will use to (1) isolate specific cell types by fluorescence-activated cell sorting (FACS) for transcriptional profiling and (2) drive the misexpression of developmentally regulated genes such as *RAMOSA3* in order to better understand the changes that occur within stem cell niches during differentiation and development. Data on the characterization of our FP and LhG4 transgenic lines, including confocal micrographs, movies, and recent publications, can be found on our website, http://maize.jcvi.org/cellgenomics/index.shtml.

Quantitative Variation in Maize Kernel Row Number Is Controlled by the FASCIATED EAR2 Locus of Maize

P. Bommert [in collaboration with M. Komatsu and
H. Sakai, DuPont Crop Genetics]

Plant growth and development are dependent on meristems, stem cell populations that serve to maintain indeterminate stem cells for organ initation throughout the life cycle. Although we know a lot about the pathways that control stem cell maintenance and proliferation, little is known about how variation in these pathways might contribute to quantitative variation in plant growth or yield. The variation in kernel rows in maize is controlled by the number of axillary meristems that are formed on the flanks of the inflorescence meristem (IM), which is located at the tip of the developing ear primordia. Many studies have identified small regions in the genome for maize kernel row number (KRN), but the genes within those regions and the molecular mechanism underlying this variation are largely unknown. Kernels are initiated following initiation and development of axillary spikelet pair meristems (SPMs), which divide once to form two spikelet meristems (SMs), each of which gives rise to floral meristems that will produce a single kernel after pollination. A simple hypothesis is that an

increase in the size of the IM would provide more available space for SPM initiation, and hence a higher KRN. Indeed, when we compared two standard inbred lines of maize, B73 and Mo17, we found that B73, which has a higher KRN, also has a larger IM. To further test our hypothesis, we dissected immature ear primordia from a number of maize inbred lines and measured IM diameter. The mean IM diameter varied between lines from ~170 μm to ~340 μm, and mean KRN varied from 10 to 20 rows. We found a significant positive correlation between IM diameter and KRN, in which variation in IM diameter explained ~60% of the variation in kernel row number. To understand the genetic contribution to the variation in maize KRN, we performed quantitative trait locus (QTL) mapping. We grew the intermated B73xMo17 (IBM) population in 3 successive years. KRN was counted from ears from ~200 recombinant inbred lines per year. The mean KRN for the parental lines was 16 for B73 and 12 for Mo17, and in the RILs, the mean KRN was 14. QTL positions were mapped using composite interval mapping. One QTL on chromosome 4 was found in each year and explained ~6%–8.5% of the KRN variation, with a positive effect from the B73 allele. Interestingly, this region contains a candidate gene, *FASCIATED EAR2* (*FEA2*). *fea2* null mutants have a significant impact on the kernel row number; the ears are highly disorganized with a massively overproliferated IM and up to 40 or more irregular kernel rows. *FEA2* encodes the maize ortholog of CLAVATA2 (CLV2), a leucine-rich repeat receptor-like protein that is involved in regulation of stem cell number by transmitting signals from the CLAVATA3 peptide ligand to repress the transcription of the WUSCHEL homeodomain transcription factor. We further substantiated these findings by isolating new, weak alleles of *fea2* that would allow us to ask if subtle variation in *FEA2* might affect KRN. Each of the newly identified alleles was predicted to be weak, and indeed, the plants homozygous for these alleles did not show ear fasciation. However, careful analysis of row number in plants homozygous for those alleles revealed subtle changes. Whereas the majority of W22 inbred ears had 14 or 16 rows, three of the weak allele populations had a significant number of homozygous mutant plants developing ears with 18–20 kernel rows. These results confirm that a weak allele of *fea2* can enhance KRN without leading to a strong fasciated phenotype that would normally be deleterious to yield. In collaboration with Dupont Pioneer, we are in the process of introducing our weak *fea2* alleles into commercial elite hybrid lines to ask whether the observed increase in KRN can be maintained and used to increase yield.

The Regulation of Meristem Size in Maize

P. Bommert, M. Pautler, B.I. Je, H. Szilagyi, L. Marshall, A. Eveland [in collaboration with M. Komatsu and H. Sakai, DuPont Crop Genetics]

All plant organs derive from populations of stem cells called meristems. These stem cells have two purposes: to divide to replace themselves and to give rise to daughter cells, which will differentiate into lateral organs. Consequently, plant meristems must precisely control the size of the stem cell niche via a network of positive and negative feedback signals. A loss of function in a negative regulator of stem cell fate can result in an enlarged or fasciated meristem phenotype and a dramatic alteration in the morphology of the maize ear and tassel.

Maize is an excellent genetic model system because of a large collection of developmental mutants and a sequenced reference genome. Our lab has undertaken a forward genetic approach to identify key regulators of stem cell homeostasis and meristem size. Two previously cloned mutants, *fasicated ear2* and *thick-tassel dwarf1*, encode orthologs of the *Arabidopsis thaliana* genes *CLAVATA1* and *CLAVATA2*, suggesting that the well-known *CLAVATA-WUSCHEL* regulatory feedback loop is conserved from dicots to monocots. However, little else is known about the control of this important developmental process in maize. Here, we describe our progress in identifying additional genes contributing to stem cell niche homeostasis.

Compact plant2 (*ct2*) is a classical mutant of maize that exhibits a fasciated inflorescence phenotype as well as semidwarfism. We discovered via a map-based cloning approach that *ct2* encodes the α subunit of a heterotrimeric GTPase, a membrane-associated protein involved in the transduction of extracellular signals to induce specific cellular responses by activating downstream effectors. To identify CT2-interacting proteins, we pursued immunochemistry approaches using transgenic maize lines expressing a translational fusion of CT2 and YFP under control of the endogenous CT2 promoter. In coimmunoprecipation experiments (Co-IPs), using a custom-made peptide antibody raised against FEA2 and a commercial YFP antibody, we found that FEA2 and CT2 interact, implicating heterotrimeric G-protein signaling in the CLAVATA signaling pathway.

Another fasciated ear mutant that we recently cloned is *fasciated ear3* (*fea3*), which was derived from a radiation mutagenesis screen in Russia. *fea3* shows an overproliferation of the inflorescence meristem. We cloned

the *fea3* gene using map-based cloning, and the mutant results from the insertion of a partial retrotransposon into an exon of the *FEA3* locus. We confirmed this identity by isolation of new alleles of *fea3* from an EMS-targeted mutagenesis. *FEA3* encodes a leucine-rich repeat (LRR) receptor-like protein similar to *FEA2*; however, double mutants of *fea2* and *fea3* have an additive fasciated phenotype in ear and tassel, indicating that they act in independent pathways. To further clarify these pathways, we have adopted a CLV3 peptide assay first used in *Arabidopsis*. In this assay, CLV3 peptide inhibits root growth in a *CLV2*-dependent pathway. Interestingly, even though *FEA3* is expressed in the root, like *FEA2*, the *fea3* mutant responds like wild-type seedlings to CLV3 peptide treatment, whereas *fea2* mutants are insensitive to CLV3 peptide. These data suggest that the regulation of stem cell proliferation in the maize *CLAVATA* pathway is not limited to a CLV3 peptide-dependent pathway and that the function of *FEA3* as an LRR receptor-like protein is distinct from that of *FEA2*. Future work will include characterizing the spatial expression of *fea3* and profiling the transcriptome to understand which pathways with which it interacts.

An additional similar mutant, *fea4*, is a semidwarfed maize mutant with thickened, or fasciated, ears and tassel. These phenotypes are caused by enlarged vegetative and inflorescence meristems, which have lost control of the pathways normally regulating meristem size. We had previously mapped *fea4* to a small region of chromosome 6 containing 30 genes. Sequencing of candidate genes in the reference allele revealed a bZIP (basic leucine zipper) transcription factor with an EMS-induced G→A transition causing a premature stop codon. Two additional alleles also contained mutations in the gene consistent with EMS mutagenesis. We subsequently obtained a series of transposon insertional mutants through a collaboration with DuPont-Pioneer. We have thus identified a novel gene required for the control of meristem size in maize.

We are characterizing the function of this bZIP transcription factor through several parallel approaches. We have generated YFP-FEA4 transgenic constructs under the native promoter and will analyze the transgenic plants in the coming months. In situ hybridization will also be used to visualize the spatial expression pattern of the gene. We will obtain a global picture of transcriptional changes in the mutant by constructing mRNA-Seq libraries. Additional approaches include making double-mutant combinations between *fea4* and other meristem regulation mutants. So far, these analyses have suggested that *FEA4* acts outside of the canonical meristem size regulation pathways, consistent with a role in buffering the balance between division and differentiation. Double mutants between *fea4* and the *ramosa* branching mutants suggest an interesting connection between meristem size regulation and meristem determinacy that will be further explored.

In addition, we are in the process of mapping ~50 fasciated ear mutants from nontargeted EMS mutagenesis screens. Our current approach involves creating F_2 mapping populations and rough mapping by bulked segregant analysis. We take advantage of the precise quantitative genotyping capability of the Sequenom MassArray system to look for areas of the genome that are linked to the phenotype. We have determined rough positions for several of these mutants and are proceeding with fine mapping and molecular cloning.

A Systems Approach to Elucidate Developmental Networks Controlling Inflorescence Architecture

A.L. Eveland, A. Goldschmidt, M. Pautler [in collaboration with D. Ware, Cold Spring Harbor Laboratory; E. Vollbrecht, Iowa State University; K. Morohashi and E. Grotewold, The Ohio State University]

Inflorescences bear the fruits and grains that we eat, and thus understanding the genetic and regulatory basis for how these structures are formed has clear relevance to important agronomic traits such as grain yield and harvesting ability. Inflorescence architecture among cereal crops is diverse, yet characterized by a unique morphology where florets are borne on short, determinate branches called spikelets. In maize, these spikelets are paired, an exclusive feature of the *Andropogoneae* tribe, which also includes important cereals and energy crops such as sorghum and sugarcane. Variations in inflorescence branching patterns arise from position and developmental fate of differentiating stem cell populations called meristems. The spatiotemporal actions of key developmental regulators, as well as genetic interactions between them, modulate meristem initiation and determinacy. Our goal is to understand the mechanisms by which these regulators act in our model system, maize, which will provide a knowledge base for the genetic control of branching and ultimately translation to other important crop species.

The primary objective of this work is to integrate various large-scale genomics data sets and to expand existing knowledge of genetic interactions that regulate branching during maize inflorescence development. Our data sets

represent maize male and female inflorescence primordia, tassels and ears, respectively, sampled during key developmental transitions and in perturbed genetic backgrounds. The latter includes loss-of-function mutants in three important regulators of the *RAMOSA* (*RA*) pathway, which controls stem-cell-fate decisions and the decision to branch. Using these data sets, we have established a robust system to investigate the networks that modulate branching, including characterization of precise timing of developmental events and associated spatiotemporal changes in gene expression. We integrated genome-wide mRNA-Seq data to resolve coexpression networks during key stages of maize inflorescence development and are working to expand these networks by incorporating additional data sets, such as genome-wide transcription factor (TF) occupancy profiles and *cis*-regulatory information. We use these approaches to prioritize candidate genes as potential regulators of important events in spikelet development, such as axillary meristem initiation and determinacy.

To establish a framework for studying coexpression networks, we characterized genome-wide expression signatures that coincided with specific morphological transitions during inflorescence development. We made use of known developmental marker genes and their spatiotemporal transcriptional responses to genetic perturbation to test our experimental system and establish a proxy for developmental staging. A k-means clustering approach revealed predicted expression patterns for previously characterized marker genes. For example, we showed that *RA1* and *RA3* were coexpressed during normal ear and tassel development, as predicted by their colocalized expression pattern at the base of the spikelet pair meristem (SPM). We then used this approach to identify candidate genes in SPM determinacy and spikelet meristem (SM) initiation networks, by integrating differential expression signatures from developmentally staged *ramosa* mutants. One interesting candidate for modulating SPM determinacy, *ZMM18*, an ortholog of the floral homeotic gene *PISTILLATA*, was localized to the SPM by in situ hybridization, an unexpected expression pattern given its previously described role in the later stages of flower development. We continue to mine coexpression clusters for enrichment of *cis*-regulatory elements and modules to computationally predict classes of TFs that regulate coexpressed genes. Work is currently under way to analyze loss-of-function mutations in new candidate regulators identified by profiling, as well as crosses to the *ramosa* mutants to study potential genetic interactions. In addition, we have generated mRNA-Seq data sets representing comparable inflorescence stages in sorghum and will use these data in comparative genomics analyses.

To further expand our coexpression network, we identified putative targets of the *RA1* TF on a genome-wide scale by chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) using maize transgenic *RA1*-tagged lines. Of ~1000 high-confidence targets, one-third were differentially expressed in *ra1* mutants based on our mRNA-Seq analysis. These putative targets were significantly enriched for TFs, and they included genes that confer inflorescence phenotypes when mutated. Our data also suggested that *RA1* is both an activator and repressor of gene expression, as groups of TFs were either up- or down-regulated. We continue to expand this network by integrating additional ChIP-Seq data sets. For example, analysis of ChIP-Seq data for *KNOTTED1* (*KN1*), a master regulator of meristem maintenance, revealed the potential for combinatorial binding with *RA1* at multiple loci in the maize genome. We are investigating enrichment of *cis*-regulatory elements within these regions to identify conserved binding sites and computationally predict additional TFs that may be interacting at these loci. Future work will investigate whether such binding is developmentally regulated.

Control of Branching and Determinacy in Plants

Y.G. Wolfenson, S. Goldschmidt

The *RAMOSA* (*RA*) genes in maize function to impose determinacy on axillary meristem growth; consequently, *ra* loss-of-function mutants (*ra1*, *ra2*, and *ra3*) have more highly branched inflorescences. *RA1* encodes a C2H2 putative transcription factor that appears to have had a key role in maize domestication and grass evolution. *RA2* also encodes a putative transcription factor, a LOB domain protein, whereas *RA3* encodes a predicted metabolic enzyme, a trehalose phosphate phosphatase. The disaccharide trehalose is not abundant in plant tissues and may have a regulatory role because it has been implicated in stress protection, control of sugar signaling, and regulation of photosynthetic rates. This is the first indication that trehalose has a specific developmental function. *RA3* is expressed in a localized domain at the base of axillary inflorescence meristems, and it localizes to nuclear and cytoplasmic compartments, suggesting that its effect on development is not simply metabolic. Interestingly, genetic and molecular studies suggest that both *RA2* and *RA3* are

required for proper *RA1* expression and act upstream of *RA1*. All three genes are expressed in overlapping domains; *RA2* is expressed in the axillary inflorescence meristems themselves, whereas *RA1* and *RA3* expression is only at the base of the axillary inflorescence meristems, suggesting that they may control a mobile signal that regulates meristem determinacy. The *RAMOSA* (*RA*) genes therefore define a boundary domain that surrounds the developing branch meristem, rather than being deployed in the meristem itself. These data support the hypothesis that *RA* genes may serve as mediators of signals, maybe a sugar signal, originated at the boundary domain and regulating determinacy. *RA3* itself may have a transcriptional regulatory function because it affects the expression of specific genes.

To find specific genes that are differentially expressed in the *RA*-specific domain, we are using FACS followed by mRNA-Seq. p*WUSCHEL*::RFP:NLS and pRA3::RFP:NLS reporter lines crossed to *ra* mutants are used for this purpose. The p*WUSCHEL* reporter is a marker for cells of the organizing center of the meristem. We expect that cell-specific profiling will enable identification of genes that act directly in the RAMOSA signaling pathway.

In another approach, we will use yeast one-hybrid analysis to identify transcription factors that regulate *RA3* gene transcription. The *RA3* putative promoter is cloned into a reporter vector and is integrated into the yeast genome. This strain provides a host for library screening. A cDNA library of DNA-binding proteins, which are expressed as fusions to the yeast GAL4 transcription activation domain (GAL4 AD prey proteins) will then be screened, in collaboration with Lifang Zhang (Ware lab at CSHL). After screening, further analysis of identified transcription factors will be done in order to learn more about their role in determinacy. Furthermore, testing whether *RA3* gene transcription is affected by other factors, such as sugars, could be performed in the yeast reporter system. These studies will therefore contribute additional information about *RAMOSA*-mediated determinacy pathways.

The *A. thaliana* genome contains a family of 10 genes related to *RA3*. Null mutants of all *AtTPP*s were tested and only one has an interesting phenotype that is connected to development and determinacy. Its late-flowering phenotype is accompanied with reduced size and shorter roots, and fusion of cauline leaves to the main stem. In addition, double mutants with *superman*, an *ra1* homolog, show increased indeterminacy, suggesting conservation of this developmental module. According to these findings, we have identified a potential ortholog of *RA3* in *Arabidopsis*, and we are characterizing additional alleles and double mutant combinations.

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PLANT DEVELOPMENTAL BIOLOGY, STEM CELLS, AND FLORAL BRANCHING SYSTEMS

Z. Lippman K. Jiang C. MacAlister
K. Liberatore S. Park

Plant architecture and reproductive success is driven predominantly by the number and arrangement of branches and flowers on structures called inflorescences. Inflorescences develop from small groups of pluripotent cells in growing tips called shoot apical meristems (SAMs). SAMs first give rise to leaves before transitioning to inflorescence meristems (IMs), which can produce lateral (axillary) meristems that either transition into flower-bearing shoots or differentiate directly into flowers. Inflorescences can be simple, producing only a single flower, or they can be highly complex, producing dozens of branches and hundreds of flowers. Our research focuses on deciphering the genetic and molecular programs that control the rates at which SAMs transition from a leaf-producing vegetative state to a flower-producing reproductive state. We are working under the hypothesis that evolutionary differences in the rate at which meristems transition to a reproductive state modulate inflorescence architecture and flower production, providing a basis for natural selection and the improvement of crop yields. Diversity in inflorescence architecture is based on two major growth habits. In "monopodial" plants, such as *Arabidopsis*, IMs grow continuously and initiate axillary flowers laterally. In contrast, in "sympodial" plants, IMs terminate in flowers and growth continues from a variable number of new axillary (sympodial) IMs that repeat this process to form compound inflorescence shoots. Sympodial plants produce a remarkable range of inflorescence architectures, but the basis for this diversity is unknown. We are uniting genetics with quantitative genomics to discover the genes and networks that control sympodial growth, which is found in half of all flowering plants, including most trees. We are taking advantage of tools in tomato (*Solanum lycopersicum*) to understand how meristem maturation and sympodial growth are modulated to give rise to compound, multiflowered inflorescences. Through knowledge gained in tomato, we are exploring mechanisms underlying variation in inflorescence branching in the larger nightshade (Solanaceae) family. Importantly, the Solanaceae comprise several crop and ornamental species, including potato, pepper, eggplant, petunia, and tobacco, as well as many edible wild species. Our research is therefore pertinent to both evolution and agriculture.

Sympodial Growth in Tomato

Tomato is a powerful model to study mechanisms of meristem maturation and sympodial growth because it produces several types of meristems, each of which undergoes a different rate of maturation (Fig. 1). Most importantly, tomato meristems are large, and easily dissected from surrounding leaves. The primary shoot meristem (PSM) originates from the embryo and produces eight leaves before switching to reproductive growth and terminating in an inflorescence. Upright growth continues from the reiteration of a specialized axillary meristem called a sympodial shoot meristem (SYM) that forms in the axil of the last leaf on the PSM and terminates after producing only three leaves. A new SYM then forms in the axil of the last leaf of the previous SYM, and all subsequent SYMs develop three leaves, a terminal inflorescence, and the next SYM to produce a compound vegetative shoot. Inflorescences are also compound, resulting from the zigzag reiteration of sympodial inflorescence meristems (SIMs), each of which gives rise to another SIM before terminating in a flower meristem (FM). Finally, each canonical axillary meristem produces primary, sympodial and axillary shoots, and inflorescences. Thus, tomato growth is modular, and numerous reproductive transitions throughout life give rise to a plant with hundreds of branches, inflorescences, and flowers (Fig. 2).

Tomato Inflorescence Architecture and the "Meristem Maturation Clock"

S.-J. Park, K. Jiang

Using a systems genetics approach, we have addressed two fascinating questions in plant biology: (1) What are the molecular dynamics underlying how SAMs switch from a leaf-producing vegetative state to a flower-producing reproductive state, and (2) what is the basis for

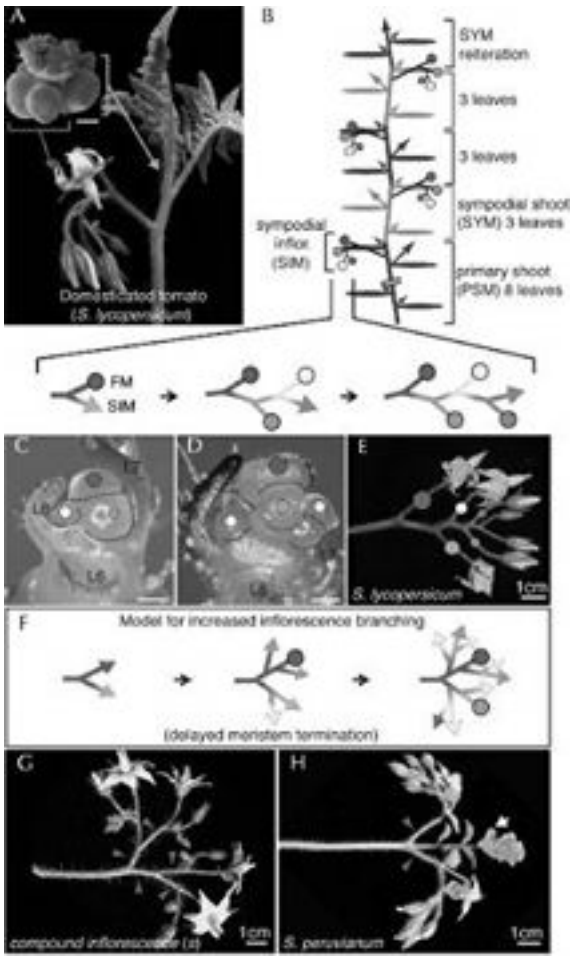

Figure 1. Sympodial growth in tomato and inflorescence architecture variation. (*A*) A tomato scanning electron microscope (SEM) image reflecting the meristems that give rise to the vegetative sympodial shoot of tomato (*right* arrow) and the multiflowered sympodial inflorescence (*left* arrow). (*B*) Schematic of tomato sympodial growth. (*C,D*) Stereoscope images of tomato meristems as they reach the reproductive state to produce a floral meristem (FM) and a sympodial inflorescence meristem (SIM). (*E*) Mature tomato inflorescence producing six to nine flowers in a zigzag pattern. (*F*) Model for inflorescence branching in the Solanaceae family based on delayed meristem maturation and termination. (*G*) Inflorescence mutant *compound inflorescence*. (*H*) Weakly branched wild tomato species, *Solanum peruvianum*.

the evolutionary diversity in inflorescence branching patterns among plants showing the widespread, yet poorly understood, "sympodial" growth habit? In the last decade, individual genes controlling inflorescence architecture have been identified; yet, the molecular programs underlying inflorescence diversity remain a mystery. By taking advantage of the large and exposed meristem of tomato, we have captured age-dependent gene expression dynamics that define the maturation of a single meristem gradually transitioning from a vegetative state to a flower. We developed a simple two-step acetone fixation technique for dissecting and collecting abundant amounts of high-quality RNA from tomato meristems. By performing deep RNA sequencing (RNA-Seq) on five stages of meristem maturation, we have revealed that thousands of genes show dynamic, age-dependent expression changes. Using these data, we applied algorithms to formulate a high-resolution quantitative "meristem maturation clock," which revealed that sympodial growth is based on primary shoot meristem maturation causing uppermost axillary meristems to adopt advanced states of maturation and terminate after brief (SYM) or no (SIM) vegetative growth.

By profiling meristems from a highly branched mutant called *compound inflorescence* (*s*), we further discovered that the primary inflorescence of *s* branches in two stages: In the first stage, multiple SIMs develop on the primary shoot due to a delay in PSM termination, and in the second stage, branching continues through delays in SIMs. In another major advance, we profile transcriptomes of meristems from a related tomato species (*S. peruvianum*), which consistently develops between two to four branches, but whose morphological variation cannot be dissected genetically. Using the meristem maturation clock, we showed that *S. peruvianum* branching is due to a surprisingly early delay in the maturation of the primary branch meristem, but a normal maturation of the SIM, thereby explaining why no more than two to four branches develop on each wild tomato species inflorescence. These findings reveal that the program for inflorescence branching is initiated surprisingly early during meristem maturation, and shows that evolutionary diversity in inflorescence architecture is modulated by heterochronic shifts in the acquisition of floral fate (Fig. 3).

Novel Inflorescence Architecture Mutants

C. MacAlister

Sympodial growth is characterized by the coordinated termination and replacement of meristems to form compound shoots. The transition from a single primary meristem to this dynamic turnover of meristems occurs at the onset of flowering, but how termination is coordinated with the formation of new meristems is not understood. By cloning genes with novel floral transition and inflorescence architecture defects, we have uncovered new pathways controlling flowering and the transition to sympodial growth.

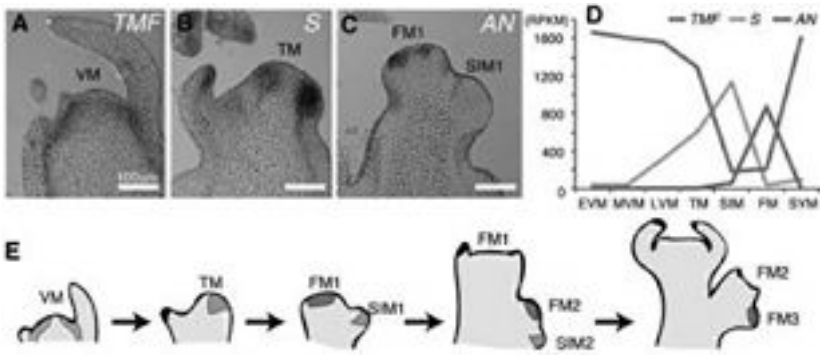

Figure 2. Transient dynamic expression of three tomato meristem maturation genes. (*A–C*) In situ hybridization of *TMF* (*A*), *S* (*B*), and *AN* (*C*). (*D*) RNA-Seq data of *TMF, S,* and *AN* revealing temporal dynamic expression. (*E*) Depiction of sequential transient expression of *TMF, S,* and *AN*. The VM expresses TMF, followed by *S* in the SIM, and *AN* in the FM. TMF expression drops as *S* is activated, and *S* drops as *AN* is activated in the *FM*. *S* followed by *AN* reiterates in subsequent SIMs to form a multiflowered zigzag inflorescence.

The classic *terminating flower* (*tmf*) mutant exhibits an accelerated transition to flowering and termination of the primary meristem in a single flower without producing any sympodial meristems to continue growth. We have cloned *TMF* and found that it encodes a protein of unknown function belonging to a small, plant-specific but deeply conserved, gene family. Furthermore, we have shown that the *TMF*-encoded protein is localized to the nucleus, and RNA-Seq and in situ hybridization reveal that the gene is expressed in the vegetative meristem before the floral transition. Using RNA-Seq, we have compared the vegetative stage meristem transcriptome of *tmf* to that of wild type. Leveraging the dynamic meristem transcriptome data generated in the lab, we have found that the genes up-regulated in *tmf* are significantly enriched for those that are most highly expressed as the meristem approaches termination, for example, the known floral meristem identity genes *FALSIFLORA* and *ANANTHA* (the tomato orthologs of the *Arabidopsis LEAFY* and *UNUSUAL FLORAL ORGANS*). Both *falsiflora* and *anantha* mutants exhibit reduced determinacy in the inflorescence and are completely epistatic to *tmf*, whereas precocious meristem expression of these genes leads to single-flowered inflorescences, as observed in *tmf*. We therefore hypothesize that *TMF* acts in the vegetative meristem to repress flowering by blocking precocious activation of the floral meristem identity program, thereby allowing sufficient time for the primary meristem to form the sympodial meristems before terminating.

Mutants in *fasciated inflorescence* (*fin*) also exhibit disruptions in inflorescence structure, but unlike *tmf, fin* mutants produce a highly branched inflorescence with greatly enlarged flowers and fruits due to a progressive expansion of the meristem over time. The *fin* phenotype is reminiscent of the *Arabidopsis clavata* mutants; in conjunction with *WUSCHEL*, the *CLAVATA* signaling cascade forms a feedback loop to maintain meristem size. By combining map-based cloning and RNA-Seq, we have cloned *fin* and found that it encodes a protein of unknown function, lacking any known protein motifs, demonstrating that additional, novel factors are involved in meristem homeostasis beyond the classic *WUS-CLV* feedback network.

Our identification of new genes controlling tomato inflorescence architecture highlights that novel mechanisms regulate determinate sympodial growth. We are now working to generate new mutants through several mutagenesis programs, as well as exploiting the phenotypic diversity found in the domesticated tomato germ plasm to identify additional players in sympodial growth.

Genetic and Molecular Characterization of Moderately Branched Inflorescence Mutants

K. Liberatore

A number of tomato accessions have strong branching inflorescences and most of this natural variation is due to mutations in the homeobox transcription factor encoded by *S*. However, the molecular basis for hundreds of weaker branching tomato variants is still unknown. To broaden our understanding of the genes and molecular networks involved in controlling inflorescence architecture, we have focused recent efforts on the mapping and characterization of several of these variants.

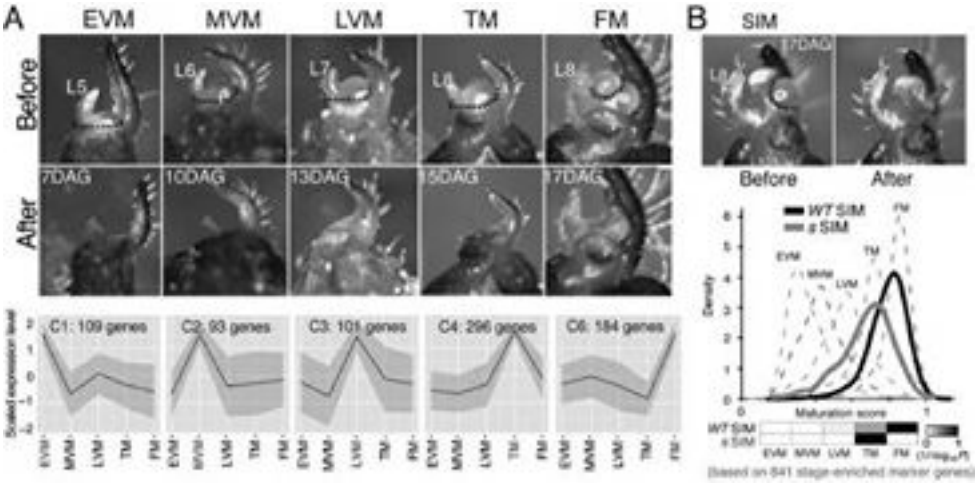

Figure 3. Characterization of tomato meristem maturation and underlying transcriptome dynamics. (*A*) Microdissection and collection of five sequential stages of the tomato primary meristem for Illumina RNA-Seq transcriptome profiling. (Dashed lines) Dissected tissue line, (arrowheads) dissected meristem, (DAG) days after germination, (L) leaf number. Shown below each image are clusters (C) of stage-enriched marker genes. (*B*) Microdissection and profiling of the uppermost axillary meristem of the primary shoot (i.e., the SIM) (*top*) and digital differentiation index (DDI) analysis predicting the maturation states of the SIM from wild-type tomato and *s* mutants. (For details, see text and Park et al. 2012.)

One group of moderately branched lines called *bifurcating inflorescence* (*bif*) branch in a manner reminiscent of *s* mutants, and preliminary allelism tests have suggested that *bif* might be a weak allele of *s*. However, because no mutations were identified in the *S*-coding region, mapping populations of three *bif* lines were generated to identify quantitative trait loci (QTL) for inflorescence branching. Interestingly, our preliminary QTL analysis on two *bif* populations revealed only a single significant QTL in the region of the *S* gene on chromosome 2, but this QTL only accounts for 25% of branching variation. These data suggest that improvement of our mapping resolution should reveal additional QTL residing elsewhere on the 12 tomato chromosomes. Further genetic and molecular characterization is necessary to determine if *S* is responsible for the chromosome 2 QTL.

We have also begun characterizing another inflorescence branching mutant called *fasciated and branched* (*fab*), which exhibits both branching and fasciation (enlargement) of the flowers and fruits. We have cloned the *FAB* gene and have found that it encodes the tomato ortholog of *Arabidopsis CLAVATA1* (*CLV1*), a well-characterized LRR receptor-like protein kinase that is involved in meristem (stem cell) maintenance. In addition to standard developmental analysis, we will further our understanding of *fab* by (1) mRNA-Seq expression profiling in *fab* mutants during the transition to flowering and (2) double-mutant analysis with other inflorescence mutants including *fin* and *s*. In particular, we are intrigued by the similar phenotypes seen in both *fab* and *fin* mutants, especially because *FIN* encodes a protein of unknown function with putative transmembrane domains, suggesting that it may also act in a receptor complex. Interestingly, the *fab:fin* double mutants result in an extremely enlarged meristem that fails to transition to flowering (sterile plants). This synergistic enhancement in the double mutant suggests that *fab* and *fin* may work in parallel pathways. We are carrying out further genetic and molecular analyses to better understand the roles of both these genes in meristem maintenance and inflorescence architecture in tomato.

Single-Gene Heterosis, Flowering, and Tomato Yield

K. Jiang

Previous studies identified a remarkable example of heterosis in domesticated tomato plants, in which heterozygosity of a single gene causes greater inflorescence production and fruit yield over both parents. Surprisingly, this "single-gene heterosis" was discovered in hybrids between homozygous mutants and nonmutant wild-type parents, suggesting that mutations can cause

overdominance. Two of the mutated genes were identified as *SFT* and *S*, which are the tomato orthologs of *Arabidopsis FT* and *WOX9*, respectively. Both genes encode key signal integrators in the pathways controlling flowering time and inflorescence architecture in plants. The nature of this single-gene heterosis led to the hypothesis that transient, semidominant expression of *SFT* and *S* in developing tomato meristems results in semidominant effects on component traits for yield, which are amplified over time due to the iterative sympodial growth pattern of tomato. We are testing this hypothesis with meristem RNA-Seq using our streamlined workflow to collect and harvest multiple meristem stages for expression profiling. Currently, for *S* heterozygous plants, RNA-Seq libraries of three key stages when semidominant expression of *S* is first evident are being profiled. These stages include transition meristems, when vegetative meristems just begin the process of committing to reproductive organ production, sympodial inflorescence meristems, a unique stage between transition and floral meristems to create the compound inflorescence of tomato, and floral meristems, which will ultimately become flowers. Preliminary analyses by semiquantitative reverse transcriptase–polymerase chain reaction (RT-PCR) have already revealed that *S* is expressed in a semidominant manner. Similar experiments are under way for *SFT* heterozygous plants, and together, these experiments should reveal the molecular basis for how mutations in flowering genes cause pleiotropic dosage effects on plant development, leading to transgressive phenotypic variation.

Interestingly, *sft* mutants may have a wild-species counterpart; the species *Solanum cheesmanii* from the Galápagos Islands is very late flowering and generates an excessive number of leaves in sympodial shoots, highly reminiscent of *sft* mutants. The excessive vegetative growth in both primary and sympodial shoots, as well as inflorescences, suggested that *S. cheesmanii* might contain loss-of-function genetic variation in the *SFT* gene or related genes in the *SFT* family. Preliminary analysis of an F2 QTL mapping population involving *S. cheesmanii* as a parent revealed a strong correlation among all the vegetative traits such as number of leaves in primary and sympodial shoots, and a large portion of the phenotypic variance of the population seems to be segregating in a Mendelian fashion, suggesting a single gene might control these phenotypes. Current work on this front includes characterizing the *SFT* gene family members in the *S. cheesmanii* genome, and the future construction of genetic markers to conduct an association study in the larger *S. cheesmanii* germ plasm to assess if late flowering is based on natural variation at the *SFT* locus.

PUBLICATION

In Press

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EPIGENETIC INHERITANCE AND REPROGRAMMING IN PLANTS AND FISSION YEAST

R. Martienssen J. Calarco, S. Castel, A.-Y. Chang, K. Creasey, E. Ernst, P. Finigan, J.-J. Han, Y. Jacob, C. LeBlanc, A. Molla-Morales, M. Regulski, J. Ren, B. Roche, A. Schorn, J. Simorowski, C. Tang, M. Tanurdžić, U. Umamaheswari, F. Van Ex, M. Zaratiegui

Plants and fission yeast provide excellent model organisms to address the question of how epigenetic information is propagated to daughter cells, including transposon regulation, heterochromatic silencing, and gene imprinting, important both for plant breeding and for human health. We are investigating the inheritance of heterochromatic silencing and the role of RNA interference (RNAi) in the fission yeast *Schizosaccharomyces pombe* and the plant *Arabidopsis thaliana,* as well as continuing our work on *Zea mays* and the *Lemnaceae* (duckweed). In the past year, we have found that RNAi is important in determining germ cell fate as well as transposon activation in *Arabidopsis*, whereas in fission yeast, transposable elements (TEs) control DNA replication via the highly conserved centromere-binding protein B (CENP-B). Importantly, we have shown that RNA and DNA polymerase collide during replication of heterochromatin and that RNAi helps resolve this collision by releasing RNA polymerase II (pol II). Release prevents DNA damage signaling and promotes spreading of histone modification, which may provide an explanation for transcription of some noncoding RNA in higher organisms.

This year, we welcomed postdocs Almudena Molla-Morales and Benjamin Roche, graduate student Stephane Castel, and computational biologist Mark Donoghue. Graduate student Patrick Finigan obtained his Ph.D. from the Watson School of Biological Sciences, and Pierre Baduel (from Polytechnique in Paris) performed his honors research project in the lab.

We said farewell to two senior postdoctoral fellows after many enjoyable and productive years: Milos Tanurdžić accepted a faculty position at the University of Queensland, and Mikel Zaratiegui became assistant professor at Rutgers, the State University of New Jersey.

RNAi Coordinates DNA Replication and Epigenetic Modification in Heterochromatin through the Release of RNA Pol II

M. Zaratiegui, S. Castel, J. Ren, B. Roche [in collaboration with Z. Cande, University of California, Berkeley; F. Antequera, University of Salamanca, Spain; B. Arcangioli, Institut Pasteur, Paris]

One defining characteristic of epigenetic modifications is their heritability. The pericentromeres of the fission yeast *S. pombe* are composed of repressive heterochromatin and repetitive noncoding RNAs whose processing by RNAi is required for histone modification. We have previously shown that these otherwise silenced noncoding RNAs are expressed specifically during S phase, the same time at which histone marks are deposited and DNA replication occurs. To further study the role of RNAi in regulating transcription, chromatin immunoprecipitation was used to interrogate RNA pol II accumulation genome wide in wild-type and RNAi-deficient *S. pombe* throughout the cell cycle. In the absence of RNAi, pol II stalling and DNA damage occur at centromeres during DNA replication, and they require repair by homologous recombination to proceed (Fig. 1). RNAi is therefore required to coordinate DNA replication and epigenetic modification in centromeric heterochromatin. We propose that transcription of noncoding RNAs during S phase leads to the collision of RNA pol II with nearby replication forks that is resolved by processing of nascent RNAs by RNAi. This processing releases pol II and allows DNA replication to proceed, but it also functions to localize the repressive histone-modifying complex to replicating heterochromatin. The RNAi-mediated release of pol II in the context of replication may occur at euchromatic loci ge-

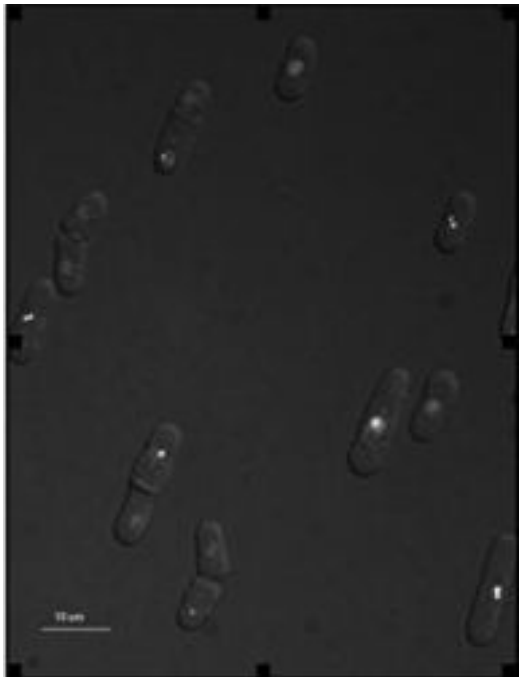

Figure 1. RNAi protects cells from spontaneous DNA damage. Rad22-YFP foci were scored under fluorescence microscopy in unsynchronized *dcr1*Δ cells, which were assessed for cell cycle stage by differential interference contrast (DIC). S phase (binucleate septated cells) could be readily distinguished from G_2 (mononuclear cells). Double foci in cells in G_2 and single foci in S-phase cells are shown (J. Ren).

nome wide and could function analogously in higher organisms. The coupling of histone modification to DNA replication may also account for the spreading of gene silencing in position-effect variegation.

CENP-B Preserves Genome Integrity at Replication Forks Paused by Retrotransposon LTR

M. Zaratiegui [in collaboration with J. Bähler, University College London; B. Arcangioli, Institut Pasteur, Paris]

CENP-B is a widely conserved DNA-binding factor associated with heterochromatin and centromeric satellite repeats. In fission yeast, CENP-B homologs have been shown to silence long terminal repeat (LTR) retrotransposons by recruiting histone deacetylases. However, CENP-B factors also have unexplained roles in DNA replication. We have shown that a molecular function of CENP-B is to promote replication fork progression through the LTR. Mutants have increased genomic instability caused by replication fork blockage that depends on the DNA-binding factor switch-activating protein 1 (Sap1), which is directly recruited by the LTR. The loss of Sap1-dependent barrier activity allows the unhindered progression of the replication fork, but it results in rearrangements deleterious to the retrotransposon. We conclude that retrotransposons influence replication polarity through recruitment of Sap1 and transposition near replication fork blocks, whereas CENP-B counteracts this activity and promotes fork stability. Our results may account for the role of LTR in fragile sites and for the association of CENP-B with pericentromeric heterochromatin and tandem satellite repeats.

DNA Methylation Dynamics through Pollen Development Involves Two Distinct Mechanisms to Control Epigenetic Reprogramming and Genomic Imprinting

J. Calarco, Y. Jacob, J. Simorowski, F. Van Ex [in collaboration with J. Feijo and J. Becker, Gulbenkian Institute, Lisbon; F. Berger, Temasek Institute, Singapore]

The male gametophyte of higher plants encloses two differentiated cells called the sperm cells and vegetative cell. Although separated by only two cell divisions, the cell types have very different chromatin architecture. We profiled DNA methylation of the three different types of nuclei observed through pollen maturation, from the postmeiotic precursor cell to the mature pollen grain, in an attempt to understand epigenetic reprogramming during plant germline development. CG and CHG methylation is reduced in the vegetative cell compared with that of sperm and microspore, but only at specific loci, which tend to mark transposable elements surrounding some noted imprinted genes. This indicates that symmetric methylation in the microspore is maintained during DNA replication in the first pollen division and actively lost at imprinted loci. In contrast, CHH methylation, which is tied to RNA-directed DNA methylation (RdDM), is much lower in sperm cells than in the companion cell, overlapping with the accumulation of corresponding small interfering RNAs (siRNAs) and preferential expression of DRM2. All of this suggests two distinct and antagonistic mechanisms: one through targeted active demethylation and another with targeted reestablishment of methylation, combined with the surprising observation that unlike mammals, the *Arabidopsis* male germline never undergoes genome-wide erasure of DNA methylation.

Multiple Roles for Small RNAs during Plant Reproduction

F. Van Ex, K. Creasey, Y. Jacob [in collaboration with F. Berger, Temasek, Singapore; J.-P. Vielle Calzada, LANGEBIO CINVESTAV, Mexico]

Germline development and early embryogenesis in eukaryotes are characterized by large-scale genome reprogramming events. In companion cells of the *Arabidopsis* male gametophyte, epigenome reorganization leads to loss of heterochromatin and production of a distinct small RNA (sRNA) population. A specific class of sRNAs derived from transposons appears to be mobile and can accumulate in germ cells. In the germline of maize, rice, and *Arabidopsis,* specific ARGONAUTE–sRNA silencing complexes appear to have key roles in reproductive development, including meiosis and regulation of germ cell fate. These results reveal new roles for sRNAs during plant reproduction and suggest that mobility of sRNAs could be critical for some of these functions. In the ovule, ARGONAUTE 9 (AGO9) has a role not only in silencing TE in the egg cell, but also in preventing the formation of multiple asexual gametophytes (Olmedo-Monfil et al., *Nature 464:* 628 [2010]). We propose that reprogramming of heterochromatin in germline companion cells reveals TE in a controlled manner to expose them within the germline and, by the production of siRNA, ensures TE silencing in the next generation. We also propose that the mechanisms evolved to silence TE may actually promote sexual reproduction by inhibiting the formation of asexual gametes.

A Functional Phylogenomic View of the Seed Plants

F. Van Ex [in collaboration with D. Stevenson, New York Botanical Garden; W.R. McCombie, Cold Spring Harbor Laboratory; G. Coruzzi, New York University; R. Desalle, American Museum of Natural History, New York]

We have used 22,833 sets of orthologs from expressed sequence tag (EST) and genome sequences from 101 genera across land plants to reconstruct their phylogenetic relationships. In using this novel phylogenomic approach, we were able to identify overrepresented functional gene ontology categories in genes that provide positive branch support for major nodes, prompting new hypotheses for genes associated with the diversification of angiosperms. RNAi has had a significant role in the divergence of monocots from other angiosperms, which has experimental support in *Arabidopsis* and rice. This analysis also implied that the second largest subunit of RNA polymerase IV and V (NRPD2) had a prominent role in the divergence of gymnosperms. This hypothesis is supported by the lack of the 24-nucleotide siRNA in conifers, the maternal control of small RNA in the seeds of flowering plants, and the emergence of double fertilization in angiosperms. Our approach takes advantage of genomic data to define orthologs, reconstruct relationships, and narrow down candidate genes involved in plant evolution within a phylogenomic view of species' diversification.

Transposon Instability in Interspecies Hybrids Is Mediated by RNAi

M. Tanurdžić, P. Finigan, E. Ernst [in collaboration with L. Comai, University of California, Davis; B.C. Meyers, University of Delaware; R.W. Doerge, Purdue University]

Silencing of transposable elements ensures genome stability and lowers the mutagenic effects of transposition. We have used statistical analysis of small RNA populations to demonstrate that COPIA retrotransposons from the pollen parent are activated in *Arabidopsis* interspecific hybrids, but only when matching siRNA are absent from the seed parent. EASY RIDER elements lose DNA methylation, and hybrid leaves accumulate the 21-nucleotide "epigenetically activated siRNA" (easiRNA) normally found in pollen, along with RNA and extrachromosomal cDNA transposition intermediates. Our results indicate that small RNA provides a general recognition mechanism for transposons in interspecific hybrids, reminiscent of hybrid dysgenesis and hybrid lethality in *Drosophila*, and may contribute to postfertilization hybridization barriers in a similar way.

cis- and *trans*-Regulation of Nonadditive Gene Expression in *Arabidopsis* Allopolyploids

P. Finigan, M.Tanurdžić [in collaboration with M. Vaughn, Cold Spring Harbor Laboratory; R.W. Doerge, Purdue University]

Allopolyploids are hybrid species that contain two or more complete chromosome sets derived from cross-fertilization of distinct species and exhibit novel phenotypic variation. This novel variation is accompanied by nonadditive gene expression whereby homologous genes (homeoalleles) are expressed at levels that differ

from the average level in the progenitor species. We have used transcriptome sequencing and high-throughput single-nucleotide polymorphism (SNP) genotyping in *Arabidopsis* allopolyploids to determine the contribution of homeoallele bias to nonadditive gene expression. We have found that homologous genes with a biased transcript accumulation in favor of one homeoallele are no more likely to be nonadditively expressed than those genes without such a bias. Furthermore, chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) indicates that homeoallele-specific expression is not associated with methylation of histone H3 at lysine-4 (H3K4me2) within exons, a euchromatic mark associated with gene expression. Although some parental bias is inherited in *cis*, we conclude that nonadditive gene expression in *Arabidopsis* allopolyploids is largely a *trans* effect, consistent with the Bateson–Dobzhansky–Muller hypothesis of gene interaction in hybrids.

Role of RDR6 in the Origin of Epialleles in *Arabidopsis*

K. Creasey [in collaboration with V. Colot, Ecole Normale Superieur, Paris; T. Kakutani, National Institute of Genetics, Japan]

The origin of epialleles by ectopic loss or gain of DNA methylation can result in epigenetically heritable gene silencing and is widespread in plants as well as in human cancer. In *A. thaliana*, rare epialleles emerge in mutants with decreased DNA methylation (*ddm1* and *met1*). We have previously reported the loss of methylation at centromeric repeats in *ddm1* and *met1* mutants, as well as the activation of all classes of TEs. Yet, *ddm1* mutants only begin to display developmental phenotypic abnormalities after several generations of inbreeding. Following the discovery of a novel class of 21-nucleotide siRNA, known as epigenetically activated siRNA (easiRNA), corresponding to pericentromeric ATHILA retrotransposons in *ddm1* mutants, we went on to create double mutants with members of the RNA-silencing pathways. We have found that developmental phenotypes are exacerbated in *ddm1* RNA-dependent RNA polymerase 6 (*rdr6*) double mutants. We have also found that the loss of RDR6 in *ddm1* leads to the loss of easiRNA. But, like in *ddm1* single mutants, transcriptome analysis reveals the activation of the same classes of TEs in *ddm1 rdr6* double mutants. We have also found an accumulation of ectopic 24-nucleotide small RNA and hypermethylation at specific loci in *ddm1 rdr6*. We conclude that the loss of *ddm1* provides an epigenetically compromised situation whereby the activation of TEs, along with the sporadic accumulation of siRNA within the genome, can result in aberrant methylation and epiallele formation.

Transposon Small RNA Expression in the Embryonic and Trophectoderm Lineage

A. Schorn [in collaboration with W. Reik, Babraham Institute, Cambridge, United Kingdom; R.W. Doerge, Purdue University]

Epigenetic reprogramming is essential to regain pluripotency during mammalian reproduction, but it also results in transient release of TEs from heterochromatin repression. In plants, transposon release in neighboring tissues triggers the production of mobile small RNAs that are capable of inducing silencing in the generative cells (Slotkin et al., *Cell 136:* 461 [2009]). We wondered if, similarly, nurse tissues in mammals express transposon-targeting small RNAs that could ensure genome integrity in the offspring. We profiled small RNA expression from mouse embryonic stem (ES) and trophectoderm stem (TS) cells to assess the potential contribution of nurse tissues to transposon silencing in the embryonic lineage in mice. We found TEs that produce small RNAs in ES or TS cells are on average younger than the mean transposon age genome-wide. In addition, endogenous retrovirus (ERV) small RNA is particularly overexpressed in TS cells. Interestingly, ERV elements are still active in mice and have contributed to placenta evolution. Therefore, small RNA expression in the trophectoderm lineage might reflect a trade-off between the benefits of transposon domestication during eutherian evolution and the necessity of protecting the embryo from active transposition. We propose an in vivo model to test for small RNA signaling between placenta tissues and the embryo proper.

The Maize Methylome: Function, Diversity, and Inheritance

M. Regulski [in collaboration with D. Ware, J. Hicks, and W.R. McCombie, Cold Spring Harbor Laboratory; S. Tingey and A. Rafalski, Pioneer-DuPont]

The maize genome has undergone dramatic changes in sequence and organization following domestication and improvement and is one of the most diverse crop genomes. We have mapped the cytosine methylation of

two maize inbred lines, B73 and Mo17, at single-nucleotide resolution. Methylation is highest in symmetric CG (65%) and CHG (50%), rather than nonsymmetric CHH (5%) contexts, and is highly enriched in transposons, repeats, and intergenic regions but not in centromeric repeats. Within genes, symmetric CG methylation (8%) and CHG methylation (5%) are found in some exons, whereas CHH methylation is found at splice sites within introns. Alignment with RNA sequences indicates that some of these patterns are correlated with small RNA, gene expression, and alternate splicing. Diversity in cytosine methylation patterns was observed in TEs and especially in genes and was found to be largely heritable in recombinant inbred lines (RILs). However, significant deviations from heritability were observed, many of which were conserved in different RILs. We conclude that directed changes in DNA methylation, resembling paramutation, are frequent events and may account for at least some of the "hidden" variation in maize breeding programs.

The Maize Mutant *Tunicate1* Is Caused by Ectopic Expression of a MADS-Box Transcription Factor, *Zmm19*, in a Dosage-Dependent Manner

J.-J. Han [in collaboration with D. Jackson, Cold Spring Harbor Laboratory]

Pod corn was once regarded as the ancestral form of cultivated corn due to its characteristic of enclosing the kernel in glumes or chaff and was prized by pre-Columbian peoples for its magical properties. The *Tunicate1* (*Tu1*) mutant of maize is a naturally occurring dominant mutation, resulting in a striking pod corn phenotype that may be present in the ancestors of maize. In a dosage-dependent manner, we could observe not only the elongation of outer glumes, but also sex determination and branching defects in tassel and ear. We cloned *Tu1* and found that it encodes a maize MADS-box gene *Zmm19,* whose 5′ untranslated region (5′UTR) region is invaded by an unknown *mutator-like* DNA transposon. We have shown that yellow fluorescent protein (YFP)- and red fluorescent protein (RFP)-tagged *Tu1* transgenic maize plants phenocopy *Tu1*. In addition, YFP- and RFP-fused Tu1 proteins are localized to the nucleus in immature reproductive organs and glumes, suggesting that Tu1 participates in specifying a floral organ identity. Moreover, misexpression of miR172 is detected in *Tu1*, suggesting that the *Zmm19* transcription factor may target the *miR172* gene in a direct or indirect way. Interestingly, the 22-Mb region surrounding the *tu1* locus in inbred B73 was selected for finishing during the maize genome sequencing project, and we have found that insertion of the *mutator-like* element resulted in a 1.8-Mb inversion immediately upstream. This rearrangement prohibits recombination and, thus, likely accompanied the origin of this ancient gene.

Biological Design of *Lemnaceae* Aquatic Plants (Duckweed) for Alkane and Biodiesel Production

E. Ernst, A. Molla-Morales [in collaboration with J. Shanklin and H. Schwender, Brookhaven National Laboratory, New York; V. Citovsky, SUNY Stony Brook]

Lemnaceae species (including *Lemna gibba*, *Lemna perpusilla,* and *Spirodela polyrhyza*) are the world's smallest aquatic flowering plants, and although they are true monocotyledonous angiosperms, they have a much reduced morphology comprising growing fronds, resting fronds, simple roots, and two "pockets" of meristematic stem cells. *Lemnaceae*, under optimal conditions, have an exponential growth rate that can double the number of fronds in 30 hours and produce 64 g of biomass per gram starting weight in 1 week, which is far beyond the fastest growing corn rates (2.3 g/g/week) and unencumbered by secondary products such as lignin. *Lemnaceae* offer an attractive alternative to algae as biofuel feedstocks, because of their robust growth in marginal aquatic environments and excellent metabolic characteristics. Existing strains of *Lemna* are already in commercial use, but they have been optimized for environmental sensing and wastewater remediation. We propose to redesign these strains for biofuel production. Our goal is to divert a substantial portion of accumulated carbon from starch to oil or alkane metabolism in *Lemna*, using resting fronds and primitive roots as the storage tissue. Clonal propagation, limited seed set, and variable chromosome number are shared with sugar cane and *Miscanthus gigantea*, and many of the technologies that we develop will have applications in other energy crops.

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In Press

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PLANT DEVELOPMENTAL GENETICS

M. Timmermans A. Benkovics, C. Clendaniel, M. Dotto, C. Fernandez-Marco, C. Hu, A. Husbands, M. Javelle, M. Lodha, K. Petsch, E. Plavskin, D. Skopelitis

Specification of adaxial–abaxial (upper/lower) polarity in the developing leaf drives the flattened outgrowth of the leaf blade and directs the differentiation of distinct cell types within the leaf's adaxial/top and abaxial/bottom domains. Both are important innovations in the evolution of land plants that maximize photosynthesis while minimizing water loss to the environment. In addition to being a key developmental process, adaxial–abaxial patterning is of particular interest from a mechanistic point of view and has proven to be an excellent model to study small RNA-mediated gene regulation. We previously showed that patterning of this developmental axis involves a cascade of opposing small RNAs, in which microRNA miR390 triggers the biogenesis of the *TAS3*-derived 21-nucleotide tasiR-ARF on the adaxial side of developing leaves. 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.

Importantly, our analysis of the *TAS3 trans*-acting small interfering RNA (tasiRNA) pathway in *Arabidopsis* demonstrated that tasiR-ARF functions as a mobile positional signal in adaxial–abaxial patterning. Movement of this small RNA from its defined source of biogenesis in the two uppermost cell layers of leaves creates a gradient of accumulation that dissipates abaxially. The tasiR-ARF gradient generates a sharply defined expression domain of the abaxial determinant ARF3 on the bottom side of leaf primordia, suggesting that small RNAs can establish pattern through a morphogen-like activity.

This work provided the first direct evidence that small RNAs are mobile and can function as instructive signals in development, thereby revealing a novel patterning activity of small RNAs.

The START Domain Regulates HD-ZIPIII Activity and Organ Polarity

A. Husbands [in collaboration with V. Yong and H. Djaballah, Memorial Sloan-Kettering Cancer Center, New York]

On the basis of our previous observations regarding the expression and function of miR166 and tasiR-ARF, it is evident that adaxial–abaxial patterning involves a cascade of positional signals. The mobile signals that pattern the newly formed leaf are distinct from those that maintain polarity during subsequent development. We have recently started a new project to identify additional signals in adaxial–abaxial patterning. Defining the identity of mobile positional signals remains not only a pressing question in the field of organ polarity, but also one of the main challenges in contemporary plant development.

The adaxial promoting HD-ZIPIII transcription factors contain a predicted START lipid-binding domain. Modeling of the START domain of the HD-ZIPIII member PHABULOSA (PHB) suggests structural similarity to human PC-TP, a phosphatidylcholine-binding START domain. This model and sequence conservation with other START domains was used to predict amino acids in PHB critical for START domain ligand binding. To assess the importance of the START domain to PHB function, we generated transgenic *Arabidopsis* lines that express START domain variants of a PHB-YFP fusion protein. These PHB-YFP reporters also carry silent mutations in the miR166 target site, which normally leads to the formation of severely adaxialized leaves. Lines expressing a PHB-YFP variant in which several residues lining the ligand-binding pocket of the START domain are mutated show a wild-type phenotype, as do lines that express a variant in which the START domain is deleted entirely. These findings reveal positive regulation of HD-ZIPIII activity by an unknown ligand and

show that the START domain is required for proper function of HD-ZIPIII proteins.

Experiments to determine the mechanism through which the START domain controls HD-ZIPIII function are ongoing. Ligand binding does not appear to affect the subcellular localization of these transcription factors, as confocal imaging showed that the PHB-YFP derivatives correctly localize to the nucleus. We are currently investigating whether ligand binding affects PHB's ability to bind DNA, to activate transcription, or to interact with required protein partners. We have also developed a yeast-based assay that allows the high-throughput screening of chemical libraries for compounds that are bound by the HD-ZIPIII START domain and modulate protein activity. Compounds identified in this screen may not only provide critical insight into the nature of the HD-ZIPIII ligand, but also identify agonists/antagonists of the START domain that enable innovative experimentation into the mechanism via which HD-ZIPIII proteins regulate diverse aspects of plant development, including stem cell activity and organ polarity. The screen is scheduled to start early in 2012.

Dissecting Small RNA Mobility in Plants

D. Skopelitis, C. Fernandez-Marco

Given the scope of miRNA-regulated gene networks, the cell-to-cell movement of small RNAs has important implications with respect to their potential as instructive signals in development or in response to physiological and stress stimuli. We are using artificial miRNAs targeting easy-to-score reporter genes to study parameters of miRNA movement, such as tissue-specificity, directionality, dose-dependence, and the kinetics of movement. Previous work provided evidence for movement of small RNAs from the epidermis into underlying cells. To further investigate miRNA mobility in multiple distinct developmental contexts, we are expressing miRNAs targeting cell-autonomous GUS and GFP reporters from a number of promoters that drive expression in various spatiotemporal patterns. A first analysis showed that miRNAs expressed from the vascular-specific *SUC2* promoter lead to miRNA-mediated gene silencing in the mesophyll, consistent with the idea that miRNAs can traffic from the vasculature into adjacent mesophyll cells. Expression of artificial miRNAs from the mesophyll-specific *RBCS* promoter leads to silencing in the pavement cells of the epidermis, indicating that miRNA mobility between the mesophyll and epidermis is bidirectional. Interestingly, no reporter silencing was observed in the stomata of the epidermis, which are symplastically isolated. This suggests that miRNAs likely traffic via plasmodesmata, channels that connect most plant cells. Analyses of miRNA mobility in other developmental contexts are still ongoing. In addition, with the knowledge that miRNAs can traffic from the epidermis into underlying tissue layers, we are carrying out forward genetic screens to identify factors influencing this process.

Additional experiments are ongoing to decipher the biological significance of small RNA mobility, particularly with respect to their role in developmental patterning. Mathematical models describing the interaction between tasiR-ARF and ARF3 predict that small RNA gradients resulting from mobility are uniquely suited to establish discreet domains of target gene expression. To test this, we are expressing a modified form of miR166 (miR166*) from promoters with distinct expression patterns in a background carrying a miR166 insensitive PHB-YFP transgene (PHB*-YFP). The miR166* sequence specifically cleaves the PHB*-YFP transcripts. We are analyzing these lines by in situ hybridization to monitor the miR166* accumulation pattern and by confocal microscopy to monitor PHB*-YFP accumulation. The outcome of these experiments will reveal whether gradients of miRNAs can sharpen target gene expression domains, which presents a novel role for miRNA mobility in pattern formation.

Characterization of Novel tasiRNA Loci in Maize

M. Dotto [in collaboration with M. Hammell, Cold Spring Harbor Laboratory; M. Aukerman, M. Beatty, and R. Meeley, DuPont-Pioneer]

tasiRNAs are processed from noncoding *TAS* precursor transcripts. Following cleavage by miRNA-loaded ARGONAUTE (AGO) complex, one of the cleavage products is converted into double-stranded RNA (dsRNA) through the activities of RNA-DEPENDENT RNA POLYMERASE6 (RDR6) and LEAF BLADELESS1 (LBL1/SGS3) and subsequently processed by DICER-LIKE4 (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 used a deep-sequencing approach to compare the small RNA content between wild-type and *lbl1* mutant apices. This identified four novel *TAS* candidates, all of which are targeted by miR390 and generate tasiRNAs closely related to the known tasiR-ARFs. We are currently using bioinformatics approaches to identify additional phased small RNA clusters in an attempt to determine whether these *TAS3* loci are the only phased siRNA generating loci in the maize genome.

In a separate approach, we are characterizing small RNAs that associate with specific AGO proteins. 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.* Phylogenetic analysis showed that the maize genome contains two close homologs of AtAGO10. We analyzed the small RNAs associated with ZmAGO10 by deep sequencing the small RNAs present in immunoprecipitates with a ZmAGO10-specific peptide antibody. We found that ZmAGO10 proteins preferentially bind 21-nucleotide small RNAs containing a 5′-U, which are features common to nearly all plant miRNAs. However, the immunoprecipitation fraction was enriched for just a subset of known miRNAs and tasiRNAs, and these include miR166 and tasiR-ARF, which we had previously shown to function in the specification of maize leaf polarity. A previously uncharacterized miRNA was most enriched in the immunoprecipitation fraction. In situ hybridization showed that this miRNA also accumulates in a polar pattern, establishing a gradient on the adaxial side of developing leaves, presenting the possibility that organ polarity is regulated also by a fourth small RNA signal. Screens for mutations affecting this miRNA and its targets are ongoing.

Small RNA-Regulated Gene Networks in Adaxial–Abaxial Patterning

K. Petsch

Mutants that disrupt tasiRNA biogenesis exhibit defects in leaf polarity, which are characterized by sectors of abaxial identity on the adaxial leaf surface and/or by radialization of the leaves. Interestingly, in maize, the severity of these phenotypes is greatly dependent on the inbred background. tasiRNA pathway mutants, as well as other small RNA biogenesis mutants, typically exhibit weaker phenotypes when introgressed into B73 as opposed to other inbred backgrounds, such as Mo17, A619, and W22. The strongest mutant phenotypes are frequently observed in W22, where severe alleles of many of the tasiRNA mutants fail to produce a shoot apical meristem. To understand how inbred background influences mutant phenotype expressivity with respect to the tasiRNA pathway, we are using the *ragged1* allele of *leafbladeless1 (lbl1-rgd1)*. Taking advantage of the different *lbl1-rgd1* phenotypes in B73 and W22, we are using an F_2 mapping population to fine-map modifiers of the pathway that either enhance the phenotype in W22 or suppress it in B73. Additionally, we have performed a transcriptome analysis of *lbl1-rgd1* mutant embryos and nonmutant siblings in B73 and W22 backgrounds. This identified 214 genes that are differentially expressed between *lbl1-rgd1* and nonmutant embryos in both backgrounds. These include known tasiRNA targets and genes showing differential expression across the adaxial–abaxial axis, as well as genes predicted to function in the embryonic shoot meristem. We are currently characterizing a select subset of differential expressed genes by in situ hybridization and reverse genetics. By combining these approaches, we hope to gain a better understanding of the factors contributing to natural variation in the tasiRNA pathway.

Adaxial–Abaxial Patterning by the AS1-AS2 Complex

A. Husbands, A. Benkovics

Our findings indicate that establishment of organ polarity requires the precise spatiotemporal accumulation and relative efficacy of tasiR-ARF and miR166. We are using genetic and biochemical approaches to identify genes that regulate the accumulation and activities of these polarizing small RNAs. We have previously shown that the *Arabidopsis* DNA-binding proteins ASYMMETRIC LEAVES1 (AS1) and AS2 form a complex that contributes to organ polarity. Using chromatin immunoprecipitation (ChIP), we have identified several polarity determinants that are direct targets of the AS1-AS2 complex. These include selected *MIR166* precursors as well as components of the *TAS3* tasiRNA pathway. Our ChIP results have been verified using reporter constructs and

reverse transcriptase–polymerase chain reaction (RT-PCR) analysis, and we are currently using ChIP-Seq to examine AS1-AS2 targets in a genome-wide context.

The abaxial determinants *ARF3* and *ARF4* are targets of the *TAS3*-derived tasiR-ARFs. We have shown that transcript levels for both *ARF* genes are up-regulated in *as2*. Considering that tasiRNA pathway components are direct targets of AS1-AS2, these transcription factors may control *ARF3* and *ARF4* expression through regulation of tasiR-ARF accumulation. To test the interaction between the AS1/AS2 and tasiR-ARF pathways, we generated *as2* plants expressing a tasiR-ARF-insensitive allele of *ARF3* (*ARF3-D*). Interestingly, double mutants develop highly serrated leaves with ectopic leaf-like outgrowths at the proximal margins. Removal of *ARF3* activity results in complete suppression of leaflet formation, as does the chemical disruption of polar auxin transport. These and other data led us to propose that leaflet formation occurs at auxin maxima in response to ARF3 activity. Confirming this, *as2 ARF3-D* plants expressing the PIN1:GFP polar auxin transport reporter show colocalization of auxin maxima with sites of incipient leaflet formation and prolonged activity of this reporter. We are currently examining the putative connection between the tasiR-ARF pathway, auxin transport and the production of compound leaf architecture.

The Ancestral Role of the tasiRNA Pathway

E. Plavskin [in collaboration with R. Quatrano, Washington University, St. Louis, Missouri; M. Hasebe, National Institute for Basic Biology, Okazaki, Japan]

The diversity of multicellular organisms raises the question of how so many varied morphologies evolved. A number of studies have demonstrated that novel structures often arise through the hijacking of existing developmental pathways for new functions. The pathways controlling leaf development seem to be no exception, as many of these have been shown to be conserved in the moss *Physcomitrella patens*, whose ancestors diverged from the lineage of flowering plants ~100 million years before leaves first evolved in the latter. Thus, studying the role of leaf polarity pathways in moss provides a unique opportunity to explore the evolution of complex novel structures.

We are focusing on the miR390-dependent tasiRNA pathway, which regulates expression of the abaxial determinants *ARF3* and *ARF4*. The genes involved in biogenesis of tasiRNAs, including *SGS3*, *RDR6*, and *DCL4*, and the tasiRNA targets are conserved between maize, *Arabidopsis*, and *P. patens*. Elucidating the function of this pathway in *Physcomitrella* may lead to an understanding of its ancestral role in plant development. To dissect the developmental processes in *P. patens* regulated by tasiR-ARFs, we are taking advantage of the unique ability of this moss to be transformed by homologous recombination and are characterizing knockouts of genes involved in tasiRNA biogenesis, as well as the targets of the moss tasiR-ARFs. Our preliminary results indicate a role for tasiRNA regulation in filamentous stem cell division in moss. *sgs3* loss-of-function mutants display increased branching in the filamentous stage of growth, and mutants of some tasiRNA targets are lethal, potentially reflecting a defect in the ability of filamentous stem cells to divide. These results are further supported by a transcriptional reporter of *miR390* in moss, which shows expression in dividing cells. Because the miR390-dependent tasiRNA pathway is also involved in meristem maintenance, our preliminary results suggest a conserved role for the pathway in plant stem cell function. We are continuing to explore the function of miR390-dependent tasiRNA biogenesis in moss through the generation of additional knockouts and expression reporters. We have also begun exploring the function of a miRNA regulatory site in tasiRNA targets, which presents the unique opportunity to study the coregulation of a gene by a tasiRNA and miRNA.

Establishment of Determinacy during Lateral Organ Development

M. Lodha

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. This project aims to dissect the genetic networks that regulate stem cell homeostasis and that distinguish indeterminate stem cells from their differentiating derivatives.

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 tran-

scription factors AS1 and AS2, which form heterodimers that bind specific sites in the *KNOX* promoters. 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*. Mutations in the PRC2 component CURLY LEAF (CLF) lead to reduced levels of this repressive H3K27me3 chromatin mark at the *KNOX* loci and to ectopic expression of *KNOX* genes in developing leaves. Likewise, H3K27me3 levels at the *KNOX* loci are dramatically reduced in the *as1* and *as2* mutants, identifying these DNA-binding proteins as upstream components in the *KNOX* silencing pathway and possible recruitment factors for the PRC2 complex. We have used bimolecular fluorescence complementation (BiFC), yeast two-hybrid and protein immunoprecipitation assays to study possible direct and indirect interactions between AS1, AS2, and PRC2 subunits. These experiments revealed multiple direct interactions between the transcription factors, AS1 and AS2, and various PRC2 subunits.

We have also established a role for the Polycomb repressive complex 1 (PRC1) in the stable silencing of *KNOX* genes during leaf development. PRC1 is a downstream component in the Polycomb pathway that maintains long-term silencing. In plants, this complex contains LIKE HETEROCHROMATIN PROTEIN1 (LHP1), which recognizes the H3K27me3 signature deposited by PRC2. We found that LHP1 directly binds silenced *KNOX* loci, and as expected, this binding is partially lost in an *as2* mutant. These data demonstrate that cellular differentiation is achieved via an epigenetic mechanism in which the AS1-AS2 complex serves to recruit Polycomb group proteins to pluripotency factors to suppress their expression during organ differentiation.

A High-Resolution Gene Expression Atlas for the Maize Shoot Apex

M. Javelle [in collaboration with M. Scanlon, Cornell University, Ithaca, New York; G. Muehlbauer, University of Minnesota, Minneapolis; J. Yu, Kansas State University, Manhattan; P. Schnable, Iowa State University, Ames]

The gene regulatory networks controlling meristem indeterminacy and organogenesis remain largely obscure. To gain insight into the gene networks involved in these distinct aspects of meristem function, we generated a high-resolution gene expression map for the maize shoot apex. Functional domains of the meristem were isolated by laser microdissection and analyzed by RNA deep sequencing. The tissues sampled are as follows: the whole meristem; the stem-cell-containing SAM tip; the newly initiating leaf (P0); the epidermal layer of the SAM (L1); the subepidermal region (L2); and vasculature within the stem. Because gene networks controlling meristem maintenance and leaf development are largely interconnected, we further compared expression profiles in an ontogenic series of leaf primordia, P1, P2, and P3, as well as between the adaxial and abaxial sides of developing leaves. We identified 757 genes that showed strong differential expression between the stem-cell-containing SAM tip and the P0 and found 502 genes that were differentially regulated between the L1 and the L2. Using unsupervised K-means clustering, we were able to identify genes that specifically mark the meristem, its stem cell domain, the P0, or developing leaf primordia (Fig. 1). This comprehensive data set allows us to precisely predict genes involved in meristem maintenance, leaf initiation, and/or leaf patterning, and to assess the distinct contributions of the L1 and L2 to these processes. In situ hybridization experiments are ongoing to verify the predicted tissue specificity of selected genes.

Several known regulators of meristem indeterminacy and organ initiation are under the control of miRNAs. To gain insight into small RNA-controlled gene networks required for SAM function, we optimized the in situ hybridization protocol for detection of small RNAs. Using this protocol, we characterized the precise expression patterns of selected mature miRNAs. Each shows a distinct expression pattern, suggesting diverse contributions of small RNAs and the pathways they target to the regulation of SAM function. miRNAs appear to function predominantly during leaf initiation and leaf development, and only one miRNA has been identified to function in the stem cell region of the SAM. The annotation of miRNA families is still ongoing. This will allow us to assess expression of individual precursor genes using the gene expression atlas. These data can highlight instances of regulated miRNA biogenesis, stability, and mobility and will allow us to gain insight into nonredundant roles in meristem function. Finally, this rich resource will be used to guide functional genomics experiments to address the role of new candidate genes in meristem function.

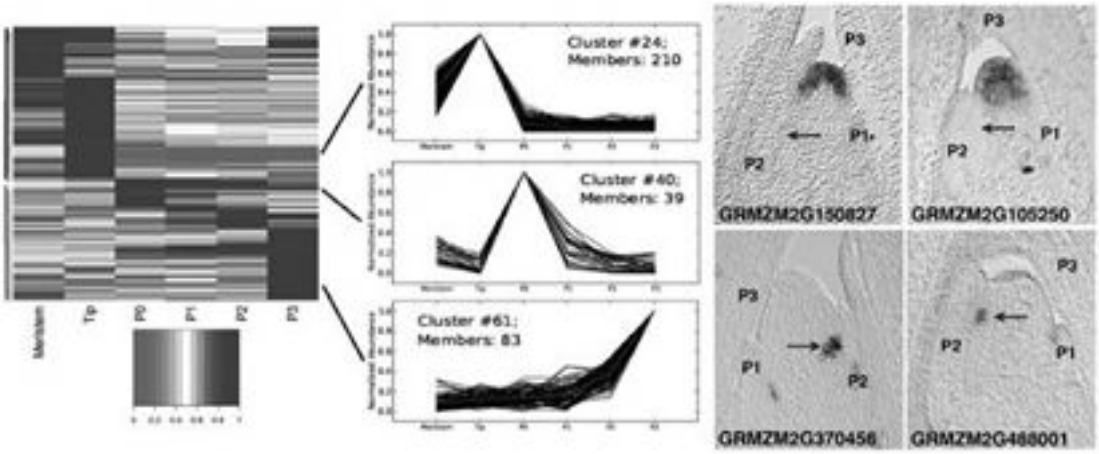

Figure 1. A high-resolution gene expression atlas for the maize shoot apex identifies genes that function in specific domains of the meristem associated with stem cell maintenance and organogenesis. Of the 18,280 genes expressed at levels above 2RPM in the whole SAM, SAM tip, and/or P0–P3 leaf primordia, 9267 are dynamically expressed, showing differential expression (≥2-fold, $q < 0.05$) in at least one of the domains analyzed. Unsupervised K-means clustering sorted these dynamically expressed genes into 74 clusters, including clusters of genes showing strong preferential expression in the meristem or in developing leaf primordia, as well as gene clusters that show a strong enrichment in the SAM tip, the P0, or specific stages of primordium development. In situ hybridization was used to validate the expression patterns of genes that, based on unsupervised K-means clustering, are predicted to localize preferentially to the SAM tip (*top right*) or P0 (*bottom right*). The arrow points to the position of the P0 leaf primordium.

Characterization of *chups*, an Embryonic Patterning Mutant in *Arabidopsis*

C. Fernandez-Marco

Embryogenesis in *Arabidopsis* proceeds through a series of highly stereotyped divisions and gives rise to a structure that contains two stem cell niches: the shoot apical meristem and the root apical meristem, which are the sources of all cells for postembryonic development. An asymmetric division of the zygote gives rise to the apical embryonic lineage and the basal extraembryonic suspensor. After several more rounds of cell division, the upper tier of the embryo 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 that wild-type embryos have reached maturity, mutant embryos show globular or heart-shaped morphologies. We have called this mutant *chupa chups* (*chups*), which means lollipop in Spanish, as a reminder of the globular shape of mutant embryos. 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*. Auxin transport and signaling are key to the specification of the hypophysis and patterning of the root stem cell niche. Preliminary data show that expression of markers for auxin transport and signaling are altered in *chups* and that an auxin maxima is no longer established at the boundary between the proembryo and suspensor, which affects specification of the hypophysal cell. Although *chups* mutant embryos are not normally viable, mutants can be rescued by transferring fertilized ovules onto culture medium. Consistent with the above-mentioned defects, rescued mutant seedlings lack a root. *chups* maps to the top arm of chromosome IV. We are currently determining the underlying mutation. The outcome of these experiments will allow us to characterize and position CHUPA CHUPS in the genetic network that patterns the root stem cell niche during embryogenesis.

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Marie Javelle

GENOMICS

Cold Spring Harbor Laboratory is at the leading edge of efforts to manage and interpret the massive amounts of information generated by contemporary biological experiments, across diverse areas of specialization. The Laboratory's commitment is seen in its ongoing participation in multinational efforts to sequence and annotate genomes of importance and in the recently established Simons 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 biological experiments.

Thomas Gingeras and colleagues study where and how functional information is stored in genomes. These efforts help to explain the biological and clinical effects of disease-causing gene mutations in humans and other organisms. Gingeras is a leader of ENCODE (ENCyclopedia of DNA Elements), the mouseENCODE, and modENCODE (model genome ENCODE) projects of the National Institutes of Health. His research has altered our understanding of the traditional boundaries of genes, revealing that almost the entire lengths of genomes in organisms ranging from bacteria to humans can be transcribed into RNA (pervasive transcription) and that most RNA products made by a cell are not destined to be translated into proteins (noncoding, or ncRNAs). In fact, ncRNAs are proving to be involved in a variety of other important biological functions. Some have been shown to be critical components in the pretranscriptional, posttranscriptional, and translational processes. Others serve as scaffolds upon which large protein complexes are assembled.

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 has been involved in international efforts culminating in genome sequences for maize and rice, two of the world's most important food crops. The lab has also had an important role in projects to sequence the flowering plant *Arabidopsis thaliana* and the fission yeast *Schizosaccharomyces pombe,* as well as *Homo sapiens* and other important genomes. McCombie's group is currently involved in several important projects to sequence genes of special interest to human health, including *DISC1*, a strong candidate gene for schizophrenia, as well as genomic regions likely implicated in bipolar illness. With the Memorial Sloan-Kettering Cancer Center, they are using a method called hybrid resequencing, developed with Greg Hannon, to look at mutations in samples collected from patients with prostate cancer.

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 and their implications for agricultural improvement. By looking comparatively across the genomes of plants in the same lineage, Ware's team seeks answers to such questions as How are genes conserved and lost over time? What are the fates of duplicated genes? The team also studies gene regulation in plants, looking at *cis*-regulatory elements and characterizing transcription factors and microRNA genes and their respective targets, with the objective of understanding how these parts of the plant genome work together in determining spatial and temporal expression of genes. The Ware lab has also had an important role in the project to produce a reference genome of maize, providing annotations and helping to generate a draft haplotype map, a gauge of maize's genetic diversity. They have devoted special attention to examining diversity within maize and grape, aiming to accelerate the development of strategies to introduce new germplasm that are needed to meet demands of an increasing population and a changing environment. The lab also has brought fully sequenced genomes into an integrated data framework to en-

hance the power of its comparative studies. This framework now includes 13 genomes of model plants such as *Arabidopsis* and important agricultural species including maize, rice, sorghum, grape, and poplar. This past year, Ware, as principal investigator for plants, helped lead an effort funded by the Department of Energy to create, out of many separate streams of biological information, a single, integrated cyber-"knowledgebase" for plants and microbial life.

GENOME ORGANIZATION, REGULATION, AND FUNCTIONAL ROLES OF NONCODING RNAS

T.R. Gingeras P. Batut, S. Chakrabortty, C. Davis, A. Dobin, J. Drenkow, M. Fastuca, S. Jha, W. Lin, J. Schlesinger, L.-H. See, H. Wang, D. Wu, C. Xue, C. Zaleski

Human/ENCODE Project

K. Bell, S. Chakrabortty, C. Davis, A. Dobin, J. Drenkow, M. Fastuca, S. Jha, W. Lin, J. Schlesinger, L.-H. See, H. Wang, C. Xue, C. Zalenski

Eukaryotic cells make many types of RNAs. As the technologies for RNA profiling and for cell-type isolation and culture continue to improve, the catalog of RNA types has grown—leading to an increased appreciation for the numerous biological roles played by RNA and arguably putting them on par with the functional importance of proteins. The Encyclopedia of DNA Elements (ENCODE) project endeavors to identify the functional elements present in the human genome sequence. One result of the pilot phase of the ENCODE project, which examined ~1% of the human genome, was the pervasive extent of transcription observed in both gene-rich and gene-poor regions, confirming some prior studies. During the second phase of the ENCODE project, the scope of examination has broadened to the complete human genome (The ENCODE Project Consortium). In this phase of ENCODE, we have sought both to provide a comprehensive genome-wide catalog of human transcripts and to identify the subcellular context for distinct RNAs and their classes. This goal was approached by identifying and characterizing annotated and novel RNAs that are enriched in either of the two major cellular subcompartments (nucleus and cytosol) for all 15 cell lines studied and, for one cell line, three additional subnuclear compartments (chromatin, nucleoplasm, and nucleoli). In addition, we have sought to determine if identified transcripts are modified at their 5′ and 3′ termini by the presence of a cap or polyadenylation and to determine as many precursor-product relationships for the identified RNAs as was possible. The long and short RNA-Seq libraries were each sequenced to an average depth of 100 million (76-mer mate-pairs) and 30 million (single-end 36-mers) reads per library, respectively. A total of 20 and 2 billion reads were mapped with splice transcript and reconstruction (STAR), a novel split read mapping algorithm or TopHat for the long and short RNA libraries, respectively. Cap analysis of gene expression (CAGE) (Djebali et al. 2012) was used to identify the 5′ end of posttranscriptionally modified transcripts and to mark transcriptional start sites (TSSs). CAGE libraries were sequenced to an average depth of ~16 million (single-end 36-mers) reads per library totaling 1.1 billion reads and were mapped with DELVE. PET (paired end tags) was used to identify 5′ ends of capped RNAs to their 3′ ends. PET libraries were sequenced to an average depth of 11.7 million (paired-end 36-mer) reads totaling ~47 million reads. These data were mapped using the TopHat algorithm. The majority of the data sets were generated in biological replicate. The raw data, mapped data, and elements are then made available by the ENCODE Data Coordination Center (DCC; http://genome.ucsc.edu/ENCODE/dataSummary.html). These data, as well as additional data on all intermediate processing steps, are available on the RNA Dashboard: http://genome.crg.cat/encode_RNA_dashboard/. This genome-wide compilation of subcellular localized and product-precursor-related RNAs serves as a public resource and reveals new and detailed facets of the RNA landscape that include the following:

- Cumulatively, a total of 62.1% and 74.7% of the human genome were observed to be covered by either processed (contigs and Gencode exons) and primary transcripts (contigs, junctions, and Gencode genes), respectively, with no cell line showing more than 56.7% of the union of the expressed transcriptome across all cells (Fig. 1).
- A consequence of the increased proportion of the human genome being transcribed is that the intergenic regions of the human genome are shrinking in size (most being <10,000 bp), having notable implications on the classic definition of a genic region.
- The current genome-wide annotated catalog of long

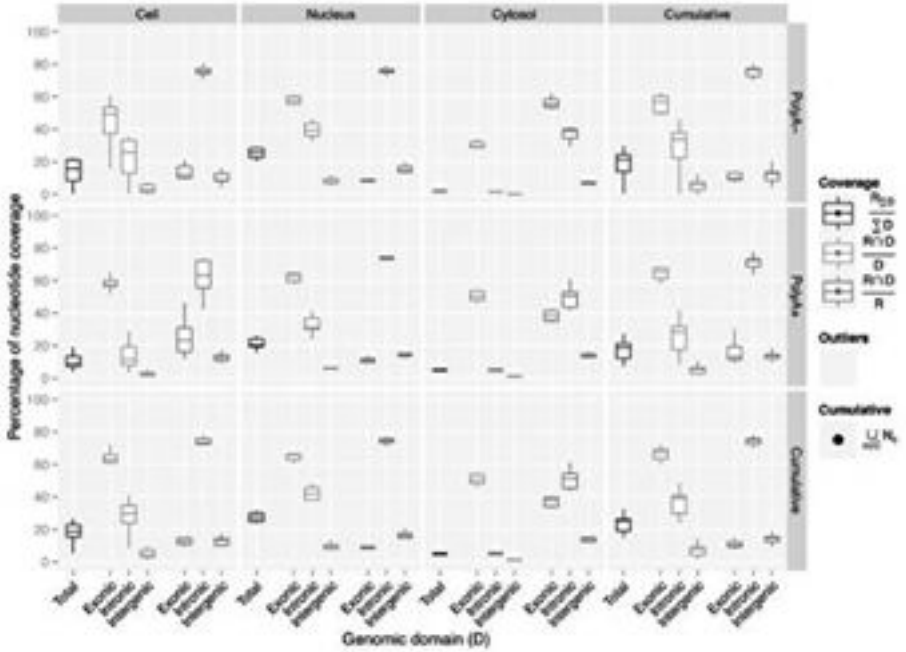

Figure 1. Nucleotide coverage of Gencode exonic, intronic, and intergenic regions. Within each cell of the plot, three different types of coverage are depicted: (1) Total nucleotide coverage of the genome (black), (2) proportion of a certain genomic domain covered by RNA-Seq reads, and (3) proportion of mapped bases that fall into a genomic domain. The box plots are generated from values across all cell lines, the largest point showing the cumulative value. These plots represent the number of nucleotides detected by RNA-Seq during the analysis of total, polyadenylated and nonpolyadenylated RNAs isolated from total cell extract and from the nucleus cytosol of the same cells. These RNAs are mapped to exonic, intronic, and intergenic regions of hg19 assembly and using the gene annotations found in Gencode v7. The last column is a cumulative summary of all coverage observed in each compartment and for each RNA subtype.

polyadenlyated and short RNAs currently cataloged by the Gencode annotation group can be increased by 94,800 exons (19%), 69,052 splice sites (22%), 73,325 transcripts (45%), and 41,204 genic regions (80%).

- Isoform expression by genes was observed not to follow a minimalistic expression strategy, resulting in genes tending to express many isoforms simultaneously, and appears to plateau at ~10–12 expressed isoforms per gene per cell line.
- Cell-type-specific enhancers are promoters that are differentiable from other regulatory regions by the presence of novel RNA transcripts, chromatin marks, and DNase-l-hypersensitive sites.

A full description of this work embodied in five manuscripts was submitted for publication recently.

Mod/ENCODE Project

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

To comprehensively characterize transcribed sequences in the genome, members of my laboratory have collaborated with many other institutions, and these are summarized in the publications listed below (Cherbas et al. 2011; Graveley et al. 2011; The medENCODE Project Consortium, *Science 330:* 1787 [2010]). RNA-Seq was performed using poly(A)$^+$ and total RNA. We found that 94% of the FlyBase (FB) 5.12 genes, and 91% of exons, showed expression in at least one of 30 developmental samples or four cell lines. The RNA-Seq data confirmed 45,153 (93%) of the 51,385 annotated FB5.12 splice junctions and identified 22,965 new splice junctions. Data from cDNA sequences, RT-PCR (reverse transcriptase–polymerase chain reaction) products, and long poly(A)$^+$ RNA-Seq support 8012 (35%) of novel splice junctions. cDNA sequences also support 236 FB5.12 gene merges and validate 485 of 1938 new predicted genes (25%). A total of 20 million CAGE tags, 1.2 million RACE (rapid amplification of cDNA ends) reads, and 50,000 expressed sequence tags (ESTs) determine embryonic transcription start sites and their distributions. A total of 12,454 promoters of 8433 genes were identified. This corresponds to 70% of the genes expressed in the embryo and 56% of the genes in FB5.12

annotations. We find that 74% of the promoters have a broad TSS distribution and 26% have a peaked TSS distribution. A total of 2075 new promoters were discovered and validated, of which 310 of the 427 promoters adjacent to active transcripts in S2 cells (72.5%) are additionally supported by promoter-associated chromatin marks discussed below, suggesting that these genes have a higher potential for alternative TSS regulation. In summary, compiling these data expanded FB5.12 by 1938 novel genes, 14,016 alternate transcripts of known genes, and 52,914 new or modified exons, of which 34,505 (65%) have been confirmed by cDNA sequencing. Furthermore, 89% of the 21,071 new exons that are expressed in S2 cells are independently supported by promoter- and transcript-associated chromatin signatures. Overall, 74% of FlyBase genes show at least one new or modified exon or alternative splice form, illustrating the importance of systematic transcriptome probing in numerous cell types, even for a species as well-studied as *Drosophila melanogaster*. These and other significant findings were recently published (Cherbas et al. 2011; Graveley et al. 2011; The medENCODE Project Consortium, *Science 330:* 1787 [2010]).

Mouse/ENCODE Project

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

This year, my laboratory joined the effort to develop a comprehensive transcription map of the mouse genome as part of the expanded ENCODE project. The work this year has focused on carrying out RNA-Seq on rRNA-depleted total RNA from 30 different mouse tissues in replicate. These data are currently being analyzed to produce mapped exon, splice site, transcript, and gene elements. Summary statistics are being compiled and cross-sample comparisons are now in preparation. The goal will be to use these mapped and summarized data in a comparative analysis with transcriptome data collected from human, fly, and worm.

Software and Database Development

A. Dobin, S. Jha, C. Xue, C. Zaleski

We currently maintain a database of sequenced libraries, including raw "fastq" data and extensive metadata. The files maintained in this database currently total more than 7 TB of storage space. The entire data set is managed by a software system constructed internally. A collection of software tools was assembled by members of our laboratory. Genomics Data Manager (GDM) is composed of a MySQL database, PHP business layer, and a web-based front end that is accessible from any browser. In addition to 7 TB of data directly managed by the database, we also maintain more than 14 TB of processed/analyzed data. An extensive software pipeline has been created to process the data through the many stages of transformation—mapping, formatting, contig generation, FPKM generation, QC stats, etc. The pipeline consists of many scripted modules, written in BASH and PYTHON, which in turn call to external analysis tools. These tools combine both internally developed and third party software. The analysis pipeline also integrates with an SGE (sun grid engine) system, using many disparate servers that range from 4 cores and 4 GB of memory up to 24 cores and 144 GB of memory.

A large proportion of these data is also sent to the CRG (Center for Genomic Regulation) in Barcelona for later submission as part of the ENCODE project. We have sent 288 libraries to the CRG comprising human long RNA (>200 nucleotides), human short RNA (<200 nucleotides), and mouse long RNA (>200 nucleotides). Each library submission contains both raw and processed data files. The CRG aggregates many varieties of data for the ENCODE Project, including CAGE, PET, MicroArray, and RNA-Seq. To date, our laboratory RNA-Seq submissions constitute the greatest proportion of submitted libraries. A timely updated status of all data comanaged by the CRG and ourselves can be found on a dashboard established by the Guigo laboratory (http://genome.crg.cat/encode_RNA_dashboard/).

Finally, recent advances in sequencing technologies have made transcriptome analyses at the single-nucleotide level almost routine. However, hundreds of millions of short (36-mer) to medium (100-mer) length sequences (reads) generated from such high-throughput sequencing experiments present unique challenges to the detection and characterization of spliced transcripts. Two key tasks make these analyses extremely computationally intensive. The first is the accurate alignment of reads that may contain mismatches caused by sequencing and mapping errors, as well as the presence of single-nucleotide polymorphisms (SNPs) and short insertion/deletions in the genome. The second task involves the mapping of reads derived from the noncontiguous regions of the genomes comprising the spliced sequence modules that are joined together to form the spliced RNAs. Although the first task is shared with DNA resequencing efforts, the second one is specific and crucial to the RNA sequencing (RNA-Seq), because it provides the connectivity information for the sequenced RNA mol-

ecules that is needed to reconstruct the full length of spliced RNA transcripts. These alignment challenges are further compounded by the presence of multiple copies of identical or related genomic sequences that are themselves transcribed, making precise mapping difficult.

An important and novel algorithm entitled STAR (a spliced transcripts alignment to the reference), which was designed to specifically address many of the challenges of RNA-Seq data mapping, was developed by Alex Dobin in our laboratory this year. This algorithm uses a novel strategy for the spliced reads alignment. STAR was designed for accurate de novo detection of the canonical splice junctions, as well as noncanonical junctions and nonlinear chimeric transcripts resulting from genomic rearrangements or *trans*-splicing. Unlike many other RNA-Seq mappers, STAR is not an extension of a short-read DNA mapper, but was developed as a stand-alone C^{++} code. STAR does not use any temporary files and hence occupies a moderate disk footprint; however, it requires a relatively large amount of RAM, ~10* (genome length) bytes, which is ~27 GB for the human genome. STAR is capable of running parallel threads on multicore systems with almost linear scaling of productivity up to 12 cores. STAR is very fast: On a modern but not overly expensive server, it can align to the human genome 36 million of 2 x 76-mer reads per hour per core, outperforming all other existing RNA-Seq aligners by more than an order of magnitude. STAR was used to map a large ENCODE transcriptome data set (>42 billion Illumina reads), and its accuracy for predicting novel splice junctions was corroborated in several high-throughput validation experiments. Approximately 82%–89% (H1ES) and 84%– 95% (HUVEC) of all the tested novel intergenic/antisense junctions supported by at least five RNA-Seq reads were corroborated by at least two amplicons sequenced by 454.

STAR was designed primarily as a tool to map shotgun RNA-Seq data to a reference genome. In certain cases, it is possible to solve the problem at hand by tweaking the alignment parameters (e.g., for mapping short RNAs or mapping long Roche 454 reads); in other cases, profound changes of the algorithm are required (e.g., for mapping Pacific Biosciences reads with high indel rate). We demonstrated STAR's ability to align long reads containing a large number of splice junction mismatches and indels by mapping the simulated Pacific Biosciences data. We have also performed successful alignments of the data from the other technologies (Ion Torrent, Roche 454, and Sanger sequencing), demonstrating that STAR can serve as a universal tool across a broad spectrum of sequencing platforms. A full description of this work has been submitted for publication recently.

Novel Exon Detection by Hierarchical RNA-Seq Clustering

K. Schlesinger, C. Davis

In part, the goals of the ENCODE transcriptome project required that we perform a large number of RNA-Seq experiments on diverse RNA samples. Integrative analysis of these experiments requires methods to normalize the data and account for varying noise levels and sensitivity. This is of special importance when trying to confidently identify novel or poorly understood classes of transcripts without much prior knowledge. We used a newly developed pool of 96 synthetic RNAs with various features covering a 2^{20} concentration range as spike-in controls to measure sensitivity, accuracy, and biases in RNA-Seq experiments as well as to derive standard curves for quantifying the abundance of transcripts. We observed linearity between read density and RNA input over the entire detection range and excellent agreement between replicates, but we observed significantly larger imprecision than expected under pure Poisson sampling errors. We use the control RNAs to directly measure reproducible protocol-dependent biases due to GC content and transcript length, as well as stereotypic heterogeneity in coverage across transcripts correlated with a position relative to RNA termini and priming sequence bias. These effects lead to biased quantification for short transcripts and individual exons, which is a serious problem for measurements of isoform abundances, but which can partially be corrected using appropriate models of bias. By using the control RNAs, we derive limits for the discovery and detection of rare transcripts in RNA-Seq experiments. Using the ENCODE data, we demonstrate that external RNA controls are a useful resource for evaluating sensitivity and accuracy of RNA-Seq experiments for transcriptome discovery and quantification. These quality metrics facilitate comparable analysis across different samples, protocols, and platforms (Jiang et al. 2011).

We also used a collection of quantified ENCODE transcriptome data to estimate the expression levels of genes on the autosomes compared to genes on the X chromosome in several male and female human cell lines. We demonstrated that the typical expression from a single active allele on an X-linked gene is identical to the typical expression from two alleles for autosomal genes for all tested classes of genes and cell lines. In collaboration with researchers at the University of Washington School of Medicine, we used these and other data to argue for "Ohno's Hypothesis" of X-chromosome dosage compensation (Deng et al. 2011).

Finally, as described previously in our work on the STAR algorithm, current high-throughput sequencing strategies rely on short reads, which represent only fragments of cDNAs. Because it is not possible to know if two different fragments came from the same transcript isoform, ab initio assembly of full-length transcripts is a complicated task with significant errors possible. This can be especially problematic in interesting loci with many different isoforms spanning a wide range of expression levels. The task is further complicated by widespread alternative splicing and many novel, poorly understood types of noncoding RNAs in complex genomes. These problems could be solved with full-length cDNA sequences from individual transcripts. Classic approaches for cDNA sequencing, however, do not provide sufficient throughput to analyze whole transcriptomes including low-abundance isoforms. We have begun to use a new approach, sequencing full-length human cDNA libraries using real-time, single-molecule, long reads from a Pacific Biosciences RS sequencer. We developed methods for library preparation, target enrichment, primary sequence analysis, and a transcript mapping pipeline. We currently can achieve about 1 million reads with average read length of more than 2500 bp, allowing us to capture many complete transcripts, including novel isoforms and noncoding RNAs, without using previous annotation or assembling across different molecules.

Transposons and Evolution of Transcriptional Regulation in the *Drosophila* Clade

P. Batut, A. Dobin

Promoters and their associated transcription start sites (TSSs) harbor the core genomic elements determining transcription. Yet their locations, features, and evolution are poorly characterized, even in well-studied model organisms, prompting large-scale efforts targeted at these critical sites. TSS discovery strategies based on 5′-complete cDNA sequencing, such as cap analysis of gene expression (CAGE), have paved the road for such endeavors, but they still suffer from very cumbersome protocols, difficult multiplexing, limited specificity for TSSs, and the acquisition of single, short sequence tags. This last point is a major limitation in two respects: It prevents the identification of the transcripts driven by a given TSS, and it precludes the study of massive regions of highly repetitive eukaryotic genomes. We developed a technology that would offer high-throughput and easy parallelization, significantly increased specificity for TSSs, and tunable sequencing read length and would allow for paired-end sequencing of 5′-complete cDNAs on the Illumina platform. We termed this technique RAMPAGE (RNA annotation and mapping of promoters for the analysis of gene expression). Benchmarking against existing techniques supports the high performance of our approach in terms of specificity for TSSs, single-base resolution, and gene expression quantification. We have designed a dedicated analysis pipeline to take full advantage of the unique features of these data. This approach was applied in a study of the dynamics of promoter activity throughout the life cycle of *D. melanogaster*. We showed that alternative promoter usage is extremely common in *D. melanogaster*, with more than 40% of genes having at least two TSSs. Alternative promoters tend to drive expression in uncorrelated patterns, suggesting that they implement different regulatory programs. Furthermore, we discovered that transposable elements harbor TSSs driving hundreds of annotated genes and often impart their own expression specificity on the genes that they regulate. In agreement with long-standing theories, our work suggests that transposons contribute significantly to the generation of standing variation and to the evolution of gene regulatory networks by distributing stereotyped regulatory modules throughout the genome. We are now applying our approach to investigate the evolutionary dynamics of promoters and their regulation throughout the *Drosophila* clade, and the role of transposons in shaping these dynamics, by conducting the same studies in other species. A complete description of this work has been recently submitted for publication.

RNA-Mediated Intercellular Signaling

S. Chakrabortty, A. Dobin

The potential role of RNAs as signaling molecules has, in part, been better understood based on studies involving cell-released exosomes. Exosomes are small 40–100-nm vesicles of endocytic origin that are secreted into the extracellular milieu on fusion of intracellular multivesicular bodies (MVBs) to the plasma membrane. Using electron microscopy, we characterized the exosomes by a cup-shaped morphology. Because of their endocytic origin, exosomes often contain both cell-surface and cytosolic proteins involved in membrane transport and fusion and MVB biogenesis. Although the mechanisms for both inclusion and uptake of exosomes containing both proteins and nucleic acids are largely undefined, extracellular exosomes are known to be enriched in raft lipids such as cholesterol, sphingolipids, and glycerophospholipids, and

the endosomal sorting complex required for trafficking (ESCRT) complex members has been implicated in sorting of the proteins to exosomes. Intriguingly, the RNA content of exosomes and their potential functional significance remain unexplored as yet. The goals of this project are to evaluate the RNA components found in mouse-tumor-derived exosomes and evaluate whether exosome delivery of these RNAs is involved in specific intercellular transference of functional long and/or short RNAs between cells. During the course of this year, several parts of this project have been addressed, including the development of an efficient and quantitative exosome isolation procedure, the validation of the purity of the isolated exosomes, RNA isolation, sequencing, characterization, and comparison of exosomal RNA and RNA from their source cells. Work has been initiated in the determination of whether exosomal-mediated transfer of the encapsulated RNA can be demonstrated and evaluation of any phenotypes associated with this transfer.

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Thomas Gingeras (*standing, center*) and lab members

DEVELOPMENT OF NEXT-GENERATION SEQUENCING TECHNOLOGY

W.R. McCombie E. Antoniou, N. Azamy, M. Bell, L. Cardone, G. Cheang, E. Ghiban, M. Gierszewska, I. Grabill, A. Karim, M. Kolli, M. Kramer, S. Lata, D. Lewis, S. Mavruk, S. McCarthy, P. Mocombe, S. Muller, M. Ossipov, J. Parla, R. Solomon, J.C. Yao

In 2011, we continued to build genomic capability, which we used both for our own lab's projects and for collaborations with other labs on campus. We produced a dramatic increase in overall sequencing capacity, shown in Figure 1. This data output increase, as well as the reduction in the cost per base that accompanies it, has been enabling for a number of projects.

In the cognitive genomics area, we have finished or are nearing completion of several projects described below, and those projects will be moving into the analysis phase. We have also optimized multiplexed exome capture and subsequent analysis for a project to study bipolar disorder and have expanded our effort to perform whole-genome sequencing (WGS) with extended families that have a high burden of psychiatric disorders as described below. Of particular interest for future progress is that we have succeeded in initial attempts to perform "microtargeting" for capturing and sequencing small regions (~0.5 million bases) from very large numbers of samples. We believe that advances in this area will be a long-term component in our studies of cognitive genomics.

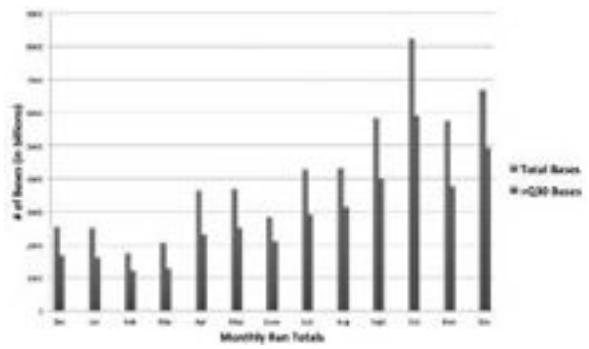

Figure 1. Comparison of total bases generated versus total high-quality bases for monthly Illumina sequencing runs from December 2010 through December 2011. We use Q30 bases as a metric to ensure very high-quality data.

In the cancer genomics field, we moved forward on two main projects. The first is a study on Barrett's esophagus and esophageal cancer in collaboration with a group at Johns Hopkins. The second is our ongoing collaboration with Memorial Sloan-Kettering Cancer Center on prostate cancer.

Lastly, we have continued our work on developing new sequencing technologies in plant genomics. This is an exciting area in which we are trying to adapt next-generation sequencing methodologies to de novo sequencing of plant genomes (Fig.1).

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, P. Thompson, I. Deary, University of Edinburgh; P. Visscher and A. McRae, University of Queensland.

Disrupted-In-Schizophrenia 1 (*DISC1*) is a strong candidate gene for bipolar disorder, schizophrenia, and cognitive disorders. A translocation disrupting this gene was first described by our collaborators in Edinburgh, who showed that it segregated significantly with psychiatric disorder in a Scottish pedigree. In conjunction with our collaborators, we initiated a pilot project to resequence ~523 kb on chromosome 1 containing *DISC1* using long-range polymerase chain reaction (PCR). The planned sequencing of the *DISC1* region on chromosome 1 in 2088 samples was completed previously and putative single-nucleotide polymorphisms (SNPs) were identified using the program MAQ (Li, *Genome Res 18:* 1851 [2008]).

After rigorous quality control, the final sample set of 1542 comprised 221 bipolar disorder, 240 schizophrenia, 192 major depression, and 889 control individuals

Table 1. Overview of *DISC1* Mutation Analysis

Category	N	% of non-rpt SNPs	% of validated SNPs	% of Exonic SNPs
SNPs not in Repeats	4243			
Minus tri-/quad-allelic and non-ref SNPs	4099	96.6		
Validated in Pile up files, AB13730 & HWE	2718	64.1		
SNPs >=0.01	708		26.0	
SNPs <0.01	2010		74.0	
Known SNPs (dbSNP) -	859		31.6	
Also in EUR 1000 genomes (ALL)	826 (1031)		30.4 (37.9)	
In regions of regulatory potential -	489		18.0	
In Conserved regions (Phastcons) -	92		3.4	
In conserved TFBS -	19		0.7	
In CpG islands -	16		0.6	
In splice sites -	3		0.1	
In exons inc UTRs -	145		5.3	
In coding regions -	34		1.3	
Synonymous SNPs -	12			35.3
Non-synonymous SNPs -	22			64.7
Predicted Stop mutations -	1*			2.9

*The stop mutation is only found in the short isoform of *DISC1*.

(the majority of controls are samples from the Lothian Birth Cohort). In 2010 and early 2011, we performed validation and initial analysis of the resulting large number of novel variants. We were able to validate 2718 rare variants by Sanger sequencing. Variants were screened to exclude repeat regions (RepeatMasker), primer regions, and triallelic regions (Table 1).

Although previous work focused on single-nucleotide variants (SNVs), in 2011 we included analysis of small insertions and deletions. The Genome Analysis Toolkit (GATK) (DePristo MA, et al. 2011) was used to generate SNPs and small insertion-deletion (indel) calls for the data. GATK was used for local read realignment around indels and for base quality score recalibration using corrections for base position within the Illumina read, sequence context, and platform-reported quality. The recalibration was followed by SNP/indel calling. Variants were filtered for a confidence score of 50, and SNP clusters (>3 SNPs per 10 bp) were excluded. Approximately 80%–90% of the SNPs called by MAQ were also called by GATK, showing a high level of concordance for the methods. With our collaborators, we will complete the analysis and validation of potentially interesting indels for this study in early 2012.

During 2011, our collaborators also performed statistical analysis of the validated SNPs, testing for association of both specific *DISC1* variants and overall burden of variants. This study uncovered a large number of novel rare variants; however, we also identified several previously reported rare coding SNPs, including R37W, which was initially reported in a patient with schizophrenia (Song et al., *Biochem Biophys Res Commun 367:* 700 [2008]). The results of the analysis indicate a possible association of noncoding SNPs with recurrent major depressive disorder. In early 2012, we will use Sanger sequencing and TaqMan assays to check for segregation of interesting rare SNVs in extended family members. We will complete final analysis of the data in 2012 and publish our findings. This study highlights rare mutations in *DISCI* that appear to contribute to psychiatric disorders.

Targeted Resequencing of the *DISC1* Interactome Using Solution-Based Hybrid Selection and Illumina Sequencing

S. McCarthy, W.R. McCombie [in collaboration with D. Blackwood, D. Porteous, I. Deary, University of Edinburgh; P. Visscher, A. McRae, University of Queensland]

The *DISC1* interactome is a network of genes that interact with *DISC1*. These genes have been implicated in psychiatric and cognitive disorders, and together with *DISC1*, they converge on pathways critical for neuronal signaling and those important in the treatment of schizophrenia, bipolar disorder, and major depression. Similar to *DISC1*, it is possible that mutations in genes constituting the *DISC1* interactome may also be of large effect and significantly increase the risk of psychiatric disorders. In collaboration with the University of Edinburgh, we are isolating and sequencing the exons, promoters, and conserved regions of 250 genes that are known to directly interact with *DISC1* or that are part of functional biological pathways related to *DISC1* interactome activity.

To overcome several technical difficulties and inconsistent data using hybrid capture arrays targeting 400 genes, and the uneven distribution of data from sequencing RainDance Technologies microdroplet PCR products spanning seven genes (*AKT1*, *GSK3B*, *NDE1*, *NDEL1*, *PDE4B*, *PDE4D,* and *TNIK*), we developed a custom solution capture probe set, in collaboration with Roche NimbleGen, to specifically enrich ~250 genes of the *DISC1* interactome. This design took advantage of progress made by Roche NimbleGen to balance probe-target selection in solution, permitting efficient custom capture of each exon, promoter, and conserved region that is 20 kb proximal and distal of all genes. Taking every isoform into account, exons of the *DISC1* interactome were obtained from RefSeq and the University of California, Santa Cruz (UCSC) gene list. Promoters were defined as those that were 2 kb upstream of each gene, whereas conserved regions were defined as runs of 10 bp or greater, with an average score equal to or greater than 0.3 based on phastCons44way nucleotide-wise conservation values.

Approximately 13 Mb (0.4%) of the human genome is selectively targeted by the custom solution capture design for the *DISC1* interactome. With such a target size, it is theoretically possible to sequence between 16 and 24 samples per HiSeq2000 lane in pools of barcoded libraries. However, we spent significant effort in testing and optimizing a pooling strategy and highlighted a number of inefficiencies with the sequence capture of whole-genome-amplified (WGA) material. Following evaluation of several pooling schemes, we determined that the most optimal strategy was to sequence 16 barcoded samples across three lanes of a HiSeq2000 flow cell.

Making use of this threefold increase in our sequence requirement, we have begun targeting the *DISC1* interactome in 1407 individuals, comprising 207 individuals with a diagnosis of bipolar disorder (BP), 225 with a diagnosis of schizophrenia (SCZ), 172 with unipolar depression (UP), 793 from the Lothian Birth Cohort (LBC), and 10 additional controls (CTRL). At the time of writing, the *DISC1* interactome had been captured in ~97% (1357/1407) of all samples WGA to preserve limited genomic DNA stocks. The remaining 3% represent samples with insufficient WGA DNA quantity or quality for capture. To date, 37.7% of the total sample (531/1407: 91 BP, 142 SCZ, 74 UP, 220 LBC, 4 CTRL) have been sequenced at a minimum depth of 20× spanning >80% of the *DISC1* interactome target. We expect to complete sequencing of all samples, analyze, and publish our results in 2012.

Sequencing of Barrett's Esophagus and Matched Adenocarcinoma Genomes

M. Streppel, S. Lata, M. Kramer, E. Antoniou, A. Maitra (Johns Hopkins Medical Institute, Maryland); W.R. McCombie

Barrett's esophagus (BE)-associated esophagus adenocarcinoma (EAC) is an aggressive tumor increasingly diagnosed in Caucasian males. The genetic basis of EAC is largely unknown. The aims of this study were to discover novel mutations during neoplastic progression of BE-EAC. Normal squamous epithelium (NSE) of the esophagus, BE, and EAC frozen biopsies were obtained from a single EAC patient and were whole-genome sequenced using Illumina next-generation sequencing in the latter half of 2010; analysis was performed in 2011. SNVs were retrieved in coding regions of annotated exons and splice sites and in regulatory and nonregulatory regions located within 2000 bp of known exons.

To identify mutations that are unique to or enriched in EAC or BE or are shared between the EAC and BE, we compared the list of EAC SNVs and BE SNVs. We identified 46 EAC-specific somatic SNVs (7 missense, 4 UTR3, 2 UTR5, 33 near gene), 50 BE-specific somatic SNVs (11 missense, 1 frame shift, 5 UTR3, 4 UTR5, 29 near gene), and 410 somatic SNVs shared between EAC and BE (64 missense, 3 nonsense, 1 splice5, 498 UTR3, 21 UTR5, and 272 near gene).

SNVs were categorized into four tiers. Tier 1 contained all SNVs in coding regions of annotated exons and splice sites, and these SNVs were validated by Sanger sequencing (n = 38). Tier 2 SNVs were located in regulatory genomic regions, tier 3 SNVs in non-repeat-masked and nonregulatory regions, and tier 4 contained all remaining SNVs. Among tier 1 SNVs, one is only present in EAC, two only in BE, and 35 in both EAC and BE. Twenty-two genes previously associated with cancer, cell cycle, and differentiation have tier 1 mutations likely to affect their function. Twenty genes with tier 1 mutations are part of a cancer-related gene network centered on *TP53* and *ITGB3*, with both genes containing mutations shared between BE and EAC.

On the basis of a mutation in one gene, our collaborators discovered that the protein from that gene is missing in 14.3% (14/98) of EAC patients. They demonstrated that this protein is involved in cellular growth and invasion of esophageal adenocarcinoma cells and that this gene regulates expression of other genes involved in regulation of cell proliferation, apoptosis, and invasion.

We are currently working to validate newly discovered short indels and structural variants and characterize their potential effects on gene function and the initiation and progression of disease.

Family-Based Analysis of Bipolar Disorder by Whole-Genome Sequencing and Target Sequencing

This work was done in collaboration with D. Blackwood, K. Evans, D. Porteous, University of Edinburgh.

A genetic study of a large Scottish pedigree by our collaborators at the University of Edinburgh suggested that a 20-Mb haplotype on chromosome 4 has significant linkage to BP (Blackwood et al., *Nat Genet 12:* 427 [1996]). We described sequencing the complete genomes of three affected and two unaffected members in a previous annual report. This work identified a small number of possible variants that segregated with the disorder. An additional 12 families, identified by our collaborators, showed linkage to the chr4 20-Mb haplotype

(Le Hellard et al., *Biol Psychiatry 61:* 797 [2007]). We sequenced the chr4 linkage region plus some of the surrounding region to allow for mapping error (36 Mb total) from multiple members of these 12 families.

We performed hybrid selection and sequencing on a 36-Mb region from 36 members of the 12 families and obtained >200x coverage on each captured individual. Reads from all flow cells for each individual sample were aligned to UCSC hg19 using Burroughs-Wheeler alignment (BWA) (Li and Durbin, *Bioinformatics 25:* 1754 [2009]). We then used PICARD (http://picard.sourceforge.net) to evaluate the quality of each library by determining the percentage of above-quality reads, mapped reads, nucleotides, paired reads, strand balance, chimeras, duplicated reads, and library complexity. SNVs/indels were called using GATK (McKenna et al., *Genome Res 20:* 1297 [2010]). The variants that we considered in analysis were restricted to those that pass GATK standard filers, and only those with at least 10-fold coverage were used in the downstream analysis. The unique SNV distribution on the chr4 36-Mb region for each of the 13 families (including the previously whole-genome-sequenced F22 family) can be found in Table 2.

We then performed a VAAST candidate-gene prioritization analysis (Yandell et al. 2011) and in-house scripts with a likelihood ratio test under the dominant-inheritance model on the basis of SNV position. Table 3 shows the VAAST candidate genes as top hits in more than one family.

We are currently selecting SNVs of potential interest from the 36-Mb region of the whole-genome sequenced family for genotyping the large cohort of case and control samples. We are also analyzing the additional 12 families to refine possible candidates.

Table 3. VAAST Candidate Genes Shown in More Than One Family

Gene	*P* value
HTT	1.01E-06
HAUS3	1.01E-06
CRIPAK	1.01E-06
SORCS2	1.01E-06
CPEB2	1.01E-06
ACOX3	1.85E-06
LGI2	3.99E-06
WFS1	2.79E-05
EVC	3.52E-05
CRIPAK	6.14E-05
C4orf48	6.14E-05
RNF212	6.14E-05
HTT	6.14E-05
OTOP1	6.14E-05

Discovering Genomic Alterations in Circulating Prostate Cancer Cells Using Whole-Exome Sequencing

This work was done in collaboration with D. Danilla, C. Sawyer, H. Scher, Memorial Sloan-Kettering Cancer Center.

Prostate cancer is the second leading cause of male cancer deaths in the United States, and most patients with metastatic castration-resistant prostate cancer (CRPC) die of their disease. Recent advances in the ability to isolate and characterize circulating tumor cells (CTCs) from the blood of CRPC patients have overcome the difficulty in obtaining sufficient metastatic CRPC tumor for genetic study. The goal of this project is to provide a comprehensive evaluation of somatic genomic alterations in circulating tumor cells in patients with CRPC.

Table 2. Unique SNV Distribution on the chr4 36-Mb Region for Each of 13 Families

SNV type	A	B	C	D	E	F	G	H	I	J	K	L	M
nonsyn	18	26	25	38	14	16	20	22	12	21	9	48	74
silent	19	30	32	26	19	11	21	26	14	11	12	66	49
stopgain	0	0	0	1	0	0	0	0	0	0	0	0	0
stoplost	1	0	0	0	0	0	0	0	0	0	1	0	1
5′ UTR	6	10	17	10	12	12	14	9	6	6	1	33	26
3′ UTR	65	56	76	50	48	41	66	44	50	42	36	102	114
splicing	0	1	1	1	0	1	1	0	0	0	0	0	0
upstream	20	46	41	50	40	18	51	58	41	12	16	131	121
downstream	47	46	58	28	38	31	46	44	35	24	18	140	128
intronic	2,559	3,934	4,284	3,446	2,878	1,958	2,945	3,736	2,203	1,918	1,863	7,865	6,151
intergenic	5,597	7,434	7,040	6,167	7,340	3,364	8,553	7,448	5,399	5,094	3,567	17,330	14,773
Ts/Tv	2.09	2.00	1.95	1.49	1.90	2.15	1.95	1.96	1.91	1.90	2.10	1.98	1.96
Her/Hom	3.21	2.74	3.85	3.73	4.59	33.37	3.38	4.44	1.60	2.39	313.39	1.41	1.16

These unique SNVs are present in the affected individuals but absent in the corresponding unaffected individuals.

We performed WGS on 31 CTC samples and 30 paired white blood cell (WBC) samples collected from 15 CRPC patients using NimbleGen exome capture (version 2). Each exome capture was sequenced in a multiplexed fashion (4-plex) in a HiSeq 2000 flow cell using paired-end 101-cycle sequencing chemistry. Before studying somatic genomic alterations in the CTC samples, we performed coverage analysis to evaluate the representation of the whole exome in each CTC/WBC sample. Only ~50% of bases on the exome were covered at 20X or greater for most of the samples. The percentage of base coverage of these exome captures can be found in Figure 2.

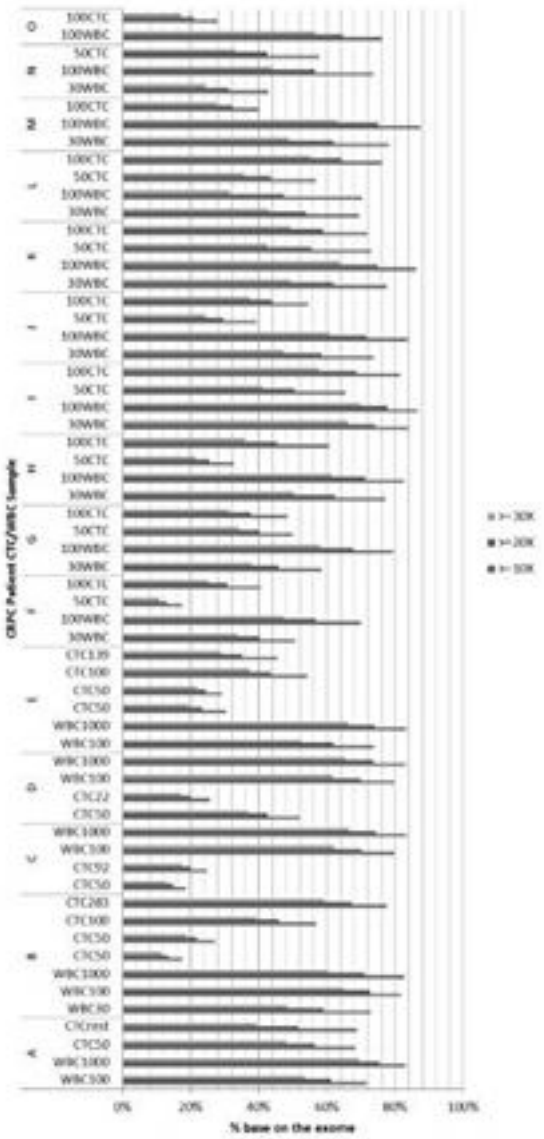

Figure 2. Percentage of base coverage on the exome for each CTC and paired WBC sample collected from 15 CRPC patients.

We then calculated the average coverage across all of the bases in each targeted region for each CTC/WBC sample and found that some regions were consistently covered at low coverage for most of the samples. For example, as shown in Figure 3, region 24830 was covered much less when compared to other regions for all of the four CTC/WBC samples obtained from one patient. This is considerably less than we see for non-CTC samples, leading us to hypothesize that the process of starting from a small number of circulating cells and greatly amplifying their DNA was causing misrepresentation in the resulting sequencing. To test this hypothesis and eliminate the possibility that the hybrid capture of the amplified DNA was introducing the bias, we performed low-pass WGS on a number of samples. WGS showed a similar pattern of uneven representation, indicating that the recovered DNA did not represent the original genome in complexity. This suggests that the exome of the sequenced CTC/WBC samples may be underrepresented.

We are now optimizing the representation of the whole exome in the CTC/WBC sample by performing a cell-number titration experiment. We are also examining the possibility of optimizing the cell-sorted and whole-genome amplification assays used for generating the CTC/WBC samples. We will continue to sequence and analyze the optimized CTC/WBC samples from additional CRPC patients to identify somatic genomic alterations. In an additional pilot study, as part of our ongoing prostate cancer project with Memorial Sloan-Kettering Cancer Center (MSKCC), we generated low-pass WGS from several tumor samples (not CTC samples). These were analyzed by MSKCC bioinformaticians and found to provide useful information about structural rearrangements, which are a common driving force in these tumors. We have started an expansion of this facet of the project with MSKCC.

Investigation of Bipolar Disorder Genetics Using Exome Capture and Resequencing

This work was done in collaboration with J. Potash (University of Iowa); P. Zandi, F. Goes, R. Karchin, A. Chakravarty (Johns Hopkins School of Medicine).

Bipolar disorder I (BPI) is a major mental illness with a significant genetic component, as demonstrated by family, twin, and adoption studies (Smoller and Finn, *Am J Med Genet C Semin Med Genet 123c:* 48 [2003]). Molecular investigation into the genetics of BPI has included linkage analyses and, more recently, several large

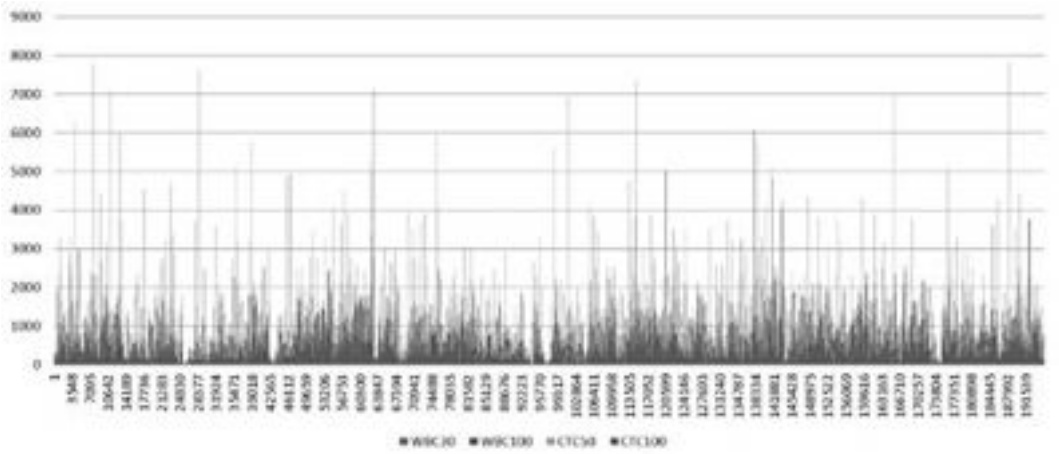

Figure 3. Average coverage across all of the bases in each targeted region for two CTC samples and two WBC samples obtained from one patient. The *x*-axis represents target regions in NimbleGen exome capture (version 2) and the *y*-axis represents the average coverage across all of the bases in each targeted region.

genome-wide association studies (GWAS) (Wendland, *Behavioral Neurobiology of Bipolar Disorder and Its Treatment, 1st ed.*, 19 [2010]). Although GWAS have yielded a small number of genome-wide significant results, the apparent "missing heritability" problem suggests that there should be rare variants contributing to BPI. Thus, approaches that are capable of effectively identifying and studying rare variants associated with BPI are necessary. We are applying exome capture and massively parallel sequencing toward finding rare genetic variants that contribute to BPI.

Our work in 2011 focused on implementing optimized lab workflows and analytical pipelines before a scale-up scheduled for 2012. To do this, we performed a pilot study of exome capture and resequencing of 201 samples, comprising 116 BPI case samples, 56 control samples, and 29 family samples (Fig. 4A). The exome capture design that we used was either the standard commercial EZ Exome version 2.0 (36-Mb genomic target) or a custom exome-plus version from NimbleGen. The custom capture product consisted of the full EZ Exome version 2.0 probes plus an additional ~6 Mb of probes to capture synaptome-specific genes and their promoters that were not already on the standard exome product. Each genomic DNA sample was prepared for capture and sequencing using Illumina TruSeq adapters that feature unique barcode bases that are sequenced independently of the library inserts, and the samples were then captured in pairs. Each captured pair was sequenced in one lane of a HiSeq 2000 PE 76 cycle run with a seven-base index read. Illumina analysis software CASAVA (version 1.7 or 1.8) was used to produce demultiplexed pass-filter sequence data, and these data were then analyzed for target coverage and SNV and indel variants using a GATK-centered pipeline established in our lab. The target coverage and library quality statistics produced from our pipeline were used to evaluate the success of the captures, for which we used a minimum threshold of 80% target coverage at ≥20× sequencing depth for a sample to successfully complete capture and sequencing (Fig. 4B). We had established this threshold in a previous study, in which we found such a criterion effective for predicting the extent of high-quality data that we should expect from a sample, but not so restrictive as to significantly limit our data or require expensive sample oversequencing to yield only small coverage benefits.

Having established a harmonized sequence data analysis pipeline between CSHL and collaborating institutions, we are finalizing a data quality control pipeline involving gender testing, principal component analysis, and Mendelian testing to evaluate the quality of our data and properly track our samples. Genetic analyses to evaluate the relationship between SNVs and indels that we identify and bipolar disorder will involve the subclassification of coding variants based on the predicted effects of the mutations on proteins, burden testing of mutation incidence in genes between cases and controls, and network- or pathway-based analyses of variants identified in cases. We also plan to complete the capture and sequencing of 1800 additional samples (predominantly cases and controls) during 2012.

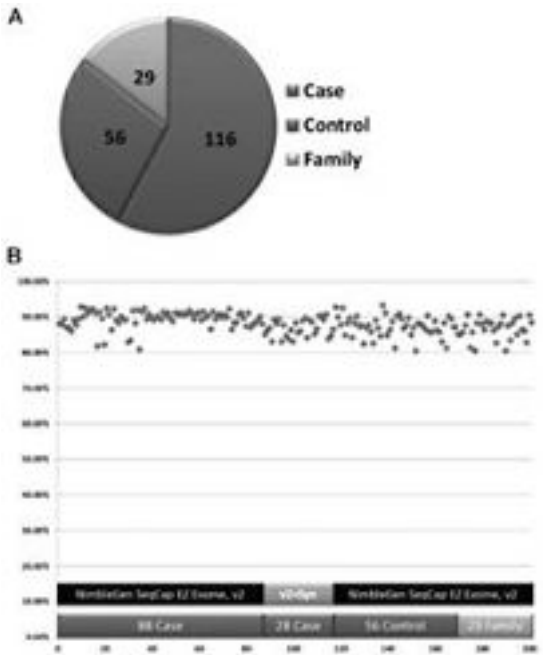

Figure 4. (*A*) Pie chart illustrating the distribution of case, control, and family samples captured and sequenced during 2011 for the bipolar disorder exome project. (*B*) Plot of percent of exome or custom exome + synaptome target covered at ≥20x sequencing depth per sample processed during 2011. Captures were performed with NimbleGen SeqCap EZ Exome version 2.0 library or NimbleGen SeqCap EZ Choice Plus custom library and sequenced using Illumina HiSeq MPE 76 x 7 x 76-cycle runs. Demultiplexed pass-filter data were analyzed for target coverage and library characteristics using a GATK-centered pipeline.

Highly Multiplexed Targeted Resequencing of Submillion Base Genomic Targets

J.S. Parla, P. Deshpande, J. Marchica, M. Kramer, E. Ghiban, S. Muller, W.R. McCombie

We and other investigators are finding potentially important candidate genes for complex traits such as psychiatric disorders through genome and exome sequencing projects. However, there is a great remaining challenge in the field to demonstrate the significance of variants in these genes or regions of the genome in very large numbers of individuals. As of now, technology does not exist that allows the sequencing of a small part of the genome (0.5–5 million bases) in large numbers of individuals (1000–10,000). We are making a focused effort to develop this capability.

To address the apparent incompatibility between genetic discovery study phases and large-scale population study phases, we have been developing a method that adapts the highly successful solution exome capture methodology to significantly smaller genomic targets. Our goals for developing our microtargeting technique include the minimal modification of existing commercial solution exome capture techniques and the establishment of maximal sample multiplexing capability at the capture level, which should support a straightforward experimental design and candidate validation strategy. We have initiated the development of our method with the commercial capture platform with which we have had the most success, namely, SeqCap EZ from Roche NimbleGen, and a selection of five human genes that were identified in a preliminary analysis of pilot exome data that we produced for one of our bipolar disorder projects. The final approved probe design targeted a cumulative 637,760 bp of the five full-length genes and was predicted to capture 92.6% of the target given a 100-base offset window accounting for library insert length.

In 2011, we initiated capture level multiplexing at three samples per capture and were able to effectively multiplex up to 15 samples per capture, based on the breadth of target coverage at ≥20X sequencing depth per sample (Fig. 5A). We empirically determined that high-quality genomic DNA input, highly sensitive double-stranded DNA–specific quantitation of the precapture library material, and the use of well-designed blocking oligos against the low-complexity sequencing-specific library adapters are important parameters for successful high-order multiplexing at the capture level. High-quality genomic DNA starting material supports the production of complex libraries that are representative of the genome, stringent DNA quantification of precapture libraries improves the distribution of samples in a capture pool (Fig. 5B), and well-designed blocking oligos significantly improve the specificity of the capture. Further development of our microtargeting method will involve increasing the sample multiplexing during capture to at least 96, improving the distribution of samples during pooling and evaluating alternative methods for precapture library preparation or target capture to improve capture performance and overall sample processing capability.

Family-Based Analysis of Schizophrenia Using Whole-Genome Sequencing

S. McCarthy, J. Badner, W.R. McCombie, W. Byerley

Interest in the contribution of rare genetic variation to the risk of psychiatric disorders has been revitalized by

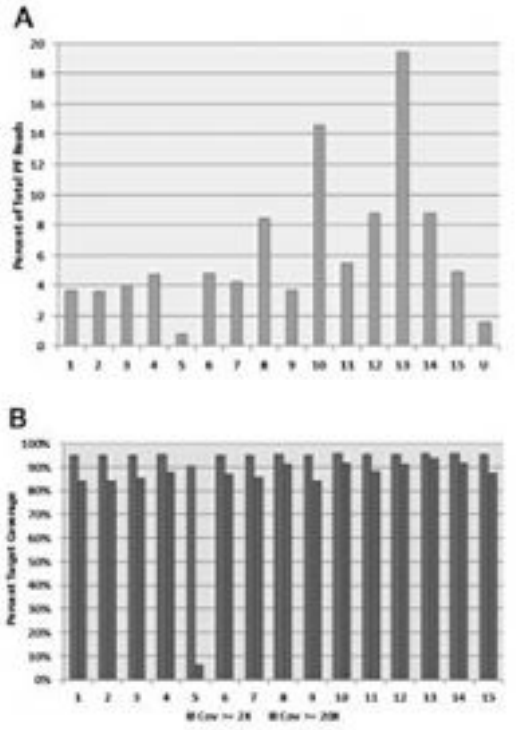

Figure 5. Multiplex capture results from a single hybridization with 15 human genomic DNA libraries and custom NimbleGen capture probes targeting five full-length genes or 637,760 bp of genomic space. Barcoded precapture libraries were prepared using NEXTflex DNA sequencing kits (Bioo Scientific) and captured according to the NimbleGen protocol, with a mixture of barcode-specific blocking oligos. The completed multiplex capture library was sequenced on one MiSeq PE 101-cycle library quality control run, which generated a total of 1.1 Gb of pass-filter sequence data. (*A*) 14 of the 15 samples produced >80% of the 637,760-bp target at ≥20x sequencing depth. Additional 15-plex captures using the same set of precapture libraries confirmed that the one sample that produced <10% target coverage at ≥20x was a result of low sequence data generated for that sample due to uneven sample distribution in the capture pool (*B*). (*B*) Perfect matches to the expected barcode sequences were required during sequence read demultiplexing, and the percent of reads matched per barcode ranged from 0.8% to 19.4%, with an average of 6.7% and a standard deviation of 4.6%. A perfect distribution would have produced 6.7% of reads per barcode. The last data point on the right (U) is the percent of reads that could not be perfectly matched to any barcode (1.6%).

recent GWAS, copy-number variation (CNV) surveys of the human genome, and rapid progress in next-generation sequencing technologies. Together with Drs. William Byerley (University of California, San Francisco) and Judith Badner (University of Chicago), we have been combining the power of WGS with the strengths of family-based analysis to uncover the genetic risk factors that potentially increase the risk of schizophrenia in two extended pedigrees. Our objective is to take advantage of WGS to catalog the genetic landscape of at least three affected individuals from two extended pedigrees with schizophrenia and prioritize genes or functional loci with rare (<1%), potentially private, high-impact variants that are shared between the affected members within each family for high throughput targeted sequencing in larger case control cohorts.

As of December 2011, we had completed WGS of 10 individuals from two extended pedigrees, K1546 and K1527. From family K1546, we sequenced an affected mother, her two affected children, her affected brother, her affected half sibling, and her unaffected husband. From family K1527, we sequenced an affected grandfather, his affected grandchild (the offspring of one of the affected children), a very distantly affected relative, and his unaffected wife. For each individual, we produced a minimum of 1.5 billion reads, of which ~90% aligned to the human hg19 reference, ensuring that more than 80% of the genome was covered at a depth of at least 30x. On average, 3.3 million SNVs and 350,000 indels were detected in each genome. Respectively, ~1% and ~5% of SNVs and indels called through our pipeline were absent from dbSNP135 and considered to be potentially novel.

Approximately 47,131 SNVs and 99,675 indels, with frequencies of <1% in the EUR population of the 1000 Genomes Project, were shared genome-wide among the affected individuals of K1546. Initially, we focused our attention on nonsynonymous SNVs (nsSNVs) in regions of linkage identified in previous genotyping scans of K1546. No nsSNVs were found within 15 Mb distal or proximal of the marker with the strongest linkage peak (2.39) on chromosome 4. However, three nsSNVs were found on chromosome 21 under a linkage peak with a LOD score of 1.34. These nsSNVs are located in three genes: *ACACB* (acetyl-coenzymeA carboxylase β), *CCDC42B* (coiled-coil domain containing 42B), and *SCARB1* (scavenger receptor class B isoform 1). The role of these genes is not clear in the neuropathology of schizophrenia, although *SCARB1* appears to be expressed in the brain, developmentally regulated, and found to be weakly associated with cognitive function in schizophrenia.

Expanding the analysis of shared nsSNVs genome-wide in K1546, ~126 variants are present, which may increase the risk of schizophrenia or diminish cognitive function in the family. One of these nsSNVs is located in the gene *DCDC2* (double cortin domain containing 2) on chromosome 6. The resulting proline-to-serine

substitution is predicted both by PolyPhen2 and VAAST to impact protein function based on nucleotide conservation and protein biochemistry. Notably, *DCDC2* was first predicted to have a role in developmental dyslexia, and in vitro and in vivo functional analyses of the gene have shown that it binds to microtubules and is involved in neuronal migration. These previous findings, coupled with the global privacy of the variant in this family, specifically in its affected members, makes *DCDC2* a strong candidate gene for follow-up analysis in larger cohorts. At present, we are validating this variant plus 231 other shared variants including splice donor and acceptor variants in all 18 members of K1546 with available DNA.

In family K1527, ~36,000 SNVs and 56,000 indels were shared among the genomes of the three affected members. However, none of the 118 nsSNVs genome-wide were in genes located under linkage peaks identified in previous linkage scans, nor were any of the nsSNVs predicted to have significant impact on gene function. It is possible that the distant relative is in fact a sporadic case of schizophrenia and consequently does not share the same risk variants as the other closer relatives. Therefore, during 2012 we shall sequence an additional relative to enrich for shared variants among relatives in family K1527 and will begin sequencing two more families with Mendelian-like patterns of inheritance of schizophrenia.

Whole-Genome Sequencing of a Consanguineous Pedigree with Bipolar Disorder and Major Depression

S. McCarthy, W.R. McCombie [in collaboration with M. Ayub (Durham University) and D. Blackwood (University of Edinburgh)]

In collaboration with Drs. Muhammad Ayub and Douglas Blackwood of the University of Edinburgh, Scotland, we are also combining the powers of WGS and family-based analysis to determine the genetic underpinnings of bipolar disorder and major depression. In contrast to ongoing family-based sequencing studies using outbred families to reduce the genetic heterogeneity of bipolar disorder and major depression, we are using a large consanguineous pedigree from the Punjab region of Pakistan to enrich for rare recessive or compound heterozygous variation of potentially large genetic effect. To achieve this goal, we generated at least 1.3 billion sequence reads for each of six individuals representing three subset inbred families of the larger consanguineous pedigree. More than 80% of each genome was covered at 30x or higher. Sequencing completed toward the end of 2011 and annotation of SNVs and indels is under way in 2012. On average, 3.4 million SNVs and 440,000 indels were found in each individual, with a slightly higher novel variation rate due to the lack of Punjab genetic variation in dbSNP135. Although our aim is to examine variants genome-wide and remain agnostic to the biology of the disorder, we will initially focus our attention on variants identified through orthogonal linkage analysis within each family and all families combined. The strongest LOD score (>10) is found on chromosome 21 and spans just 10 kb. The region was identified using FESTIM, a software package designed specifically to examine inbred pedigrees; however, prior resequencing of the region revealed little or no sequence variation. Our evaluation of the chromosome 21 region will also take the latest ENCODE data into account so that it will not be limited to just nonsynonymous or frame shift indels. In addition, we will explore the region for CNV that could also explain the desertion of SNVs previously found. In parallel to this analysis, we will examine the entire genome for shared recessive and or codominant heterozygous mutations. Such analyses, to the best of our knowledge, have not yet been explored and should make a significant contribution to our understanding of the neuropathology of bipolar and major depression.

Plant Genomics

S. Pasternak, M. Wright, C.-W. Tung, C. Jer-Ming, Y. Berstein, E. Ghiban, M. Kramer, E. Antoniou, R. Martienssen [in collaboration with S. McCouch (Cornell University)]; D. Ware, M. Schatz, W.R. McCombie

We generated several different types of sequencing libraries for several species of rice and wheat to test various ways to assemble these complex genomes.

We obtained high-level sequence coverage from three varieties of rice (Nipponbare, IR64, and DJ123) from standard Illumina libraries (300-bp insert). We also sequenced 180-bp insert libraries designed to work with the ALLPATHS software (Gnerre 2010), at 150x for Nipponbare, 130x for IR64, and 105x for DJ1232. To complement these libraries, we made Illumina mate-pair libraries and sequenced them at the following depth of coverage: 235x for Nipponbare (2-kbp insert), 180x and 145x for IR64 (2- and 5-kbp inserts), and 180x and 160x for DJ123 (2- and 5-kbp inserts). We also used the recently released Illumina MiSeq se-

quencer to generate longer 250-bp pair reads to study the potential advantage of using these reads in the assembly process, to about 8× coverage of the three rice genomes.

We used the Pacific Biosciences (PacBio) RS sequencers to obtain 5.7× genomic coverage of long single-molecule sequencing reads for the Nipponbare genome. These data are being used to evaluate the efficacy of combining PacBio and Illumina sequencing reads for genome assembly.

We sequenced two wheat genomes, *Ae. taushii* and *Triticum monococcum*, using ALLPATHS 180-bp insert libraries and paired-end 100-bp reads, at up to 69× and 71× genomic coverage, respectively. We supplemented these data with 2- and 5-kbp mate-pair libraries sequenced at 77× and 6.5× genomic coverage for *Ae. taushii* and at 66× and 9× genomic coverage for *T. monococcum*. We are currently testing, in collaboration with Mike Schatz, the assembly of these different data sets.

PUBLICATIONS

DePristo MA, Banks E, Poplin R, Garimella KV, Maguire JR, Hartl C, Philippakis AA, del Angel G, Rivas MA, Hanna M, et al. 2011. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet* **43:** 491–498.

Yandell M, Huff CD, Hu H, Singleton M, Moore B, Xing J, Jorde LB. 2011. A probabilistic disease-gene finder for personal genomes. *Genome Res* **21:** 1529–1542.

W.R. McCombie (*top row, fifth from right*) and lab members.

PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware J.-M. Chia, A. Chuah, A. Eveland, S. Kumari, Y.K. Lee, Z. Liu, Z. Lu, M.K. Monaco, C. Noutsos, A. Olson, S. Pasternak, W. Spooner, J. Stein, J. Thomason, P. Van Buren, S. Wei, K. Youens-Clark, L. Zhang

Our lab has dual goals of conducting plant genomics research and developing genomics resources and discovery tools for use by the broader research community. Research includes a broad range of activities in physical, statistical, and functional genomics in both model systems and crop plants, with applications in agriculture. During the past year, we were joined by scientist Marcela Karey Monaco and postdoctoral researcher Young Koung Lee, and we said goodbye to Aaron Chuah, Will Spooner, and Zhijie Liu.

Plant Genome Research

In the last decade, the decoding of complete plant genomes has borne fruit for scientists who seek to understand the complexities of plant function and evolution and to affect economically important traits. The generation of reference genomes spans many disciplines, starting in the laboratory with scientists who generate the raw sequence data. From there, it is the responsibility of computational biologists and bioinformaticians, such as those in the Ware lab, to help interpret the output. This includes assembly of raw sequence reads into overlapping segments, or contigs, and scaffolding these to properly represent their order and orientation within chromosomes. Another step is annotation—the discovery and description of genes and other functional elements, as well homologies to other genomes. In addition, this information must be faithfully communicated and visualized, for example, in web-based platforms such as Gramene.

All of these activities are rapidly evolving with the advent of new sequencing technologies, algorithms, and data-handling requirements. For example, high-depth sequencing of RNA transcripts at low cost is providing new evidence that informs genome annotation, and it is spurring the development of new software to model and perform this task. Low-cost sequencing is also transforming the types of questions that can be asked, moving beyond the generation of a single reference for a given species. Ongoing projects within the maize, rice, and *Arabidopsis* research communities are now sequencing hundreds and even thousands of genotypic backgrounds within species, gathered from carefully constructed populations, wild populations, and breeding germplasms. Information on genetic variation is helping scientists to understand the genetic basis of phenotypic traits and questions about the origins of domesticated crop species. New technologies are also driving research in epigenetics, the study of heritable traits that are not caused by changes in the underlying genome sequence. Epigenetic mechanisms include modification of DNA by methylation and various forms of histone modifications, which can cause changes in gene expression and other phenotypes. Both of these modification types can be studied with new sequence technologies and analysis methods. Our lab is engaged in many of these areas of inquiry. For some species such as maize, we are beginning to see how the provision of genome sequence, annotation, genetic variation, epigenetic variation, and transcript expression is converging to bring about a deeper understanding of genome function.

Gramene

A. Chuah, M.K. Monaco, S. Pasternak, W. Spooner, J. Stein, J. Thomason, S. Wei, K. Youens-Clark, S. Kumari [in collaboration with E. Buckler, USDA, Cornell University; S. McCouch, Cornell University; P. Jaiswal, Oregon State University; P. Kersey, European Bioinformatics Institute]

Gramene is a collaborative project that leverages sequence and functional information from plant reference genomes to promote research and translational genomics in agriculture and its various applications including food and biofuel production. 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 completed several milestones on the Gramene project, including its 33rd and 34th releases since 2000. Of the many improvements made, we increased the number of partial and complete sequenced genomes in our Ensembl genome browser to 22 scientifically and economically important species including rice, corn, sorghum, soybean, poplar, grape, and the unicellular green alga *Chlamydomonas reinhardtii*. Gramene is also home to reference sequences for three cultivated and eight wild species of rice, making it the single most comprehensive genus level (*Oryza*) resource in plants. During 2011, we released pathway databases for maize (MaizeCyc) and *Brachypodium* (BrachyCyc), complementing existing pathway databases for rice, sorghum, and other plants.

Gramene is also positioning itself as a principal resource for conducting population-based genetics research. We added several new diversity/phenotype data sets covering *Arabidopsis*, rice, and maize and have added new tools for querying single-nucleotide polymorphisms (SNPs) and visualizing genome-wide association studies. In addition, we used the Ensembl 64 infrastructure to include multispecies views from whole-genome alignments, synteny maps, browsable phylogenetic gene trees, and SNP views that display diversity data in the context of functional impacts on gene structure. All of this work has contributed to a 38% increase of traffic on the Gramene website during the last 2 years from users all over the world. Our databases are created in partnership with the European Bioinformatics Institute (EBI) and are now also available through the Ensembl Plant portal. During the next year, we will continue to add new reference genomes, data sets, and functionalities to this resource.

Updating the Maize Reference Genome and Annotation

A. Olson, S. Pasternak, J. Stein [in collaboration with J. Glaubitz and E. Buckler, Cornell University; R. Fulton and R. Wilson, Washington University; The Maize Genome Sequencing Consortium]

Work continues on refining the assembly and annotation of the maize cv. B73 reference sequence. The primary objective is to place genes that are currently missing or incomplete. A whole-genome shotgun library was sequenced to 14× coverage using the 454 Titanium method, and resulting reads were assembled using Abyss. To define genes, we used the FLcDNA collection, aligning both contig and RefGen_v2 sequences. A graph-based algorithm was developed to construct novel gene scaffolds having complete coding sequence coverage. The gene scaffolds were genetically mapped by a previously described genotype-by-sequencing (GBS) method, which was applied both to the 454 contigs and the FLcDNA sequences. Final incorporation of gene scaffolds into the reference sequence was refined using DNA- and gene-based synteny to rice and sorghum. The resulting new assembly contains an additional ~1.3 Mb of sequence. Approximately 500 genes were added or improved with new annotation. The GBS results, along with synteny, were also used to anchor a number of unknown (chr0) physical map contigs. Release of the new reference assembly will proceed following acceptance by GenBank.

Tackling Wheat

J. Stein, S. Pasternak, A. Olson [in collaboration with J. Dvorak and M.C. Luo, University of California, Davis; O. Anderson, University of California, Davis/USDA-ARS, Albany; B.S. Gill, Kansas State University; W.R. McCombie and M. Schatz, Cold Spring Harbor Laboratory]

One of the great frontiers in plant genomics is to determine the complete sequence of the bread wheat *Triticum aestivum*. From its early domestication in the Fertile Crescent, large-scale cultivation of wheat enabled the rise of human civilization and the formation of city-based societies. Today, it remains one of the most important food crops worldwide, grown on more land area than any other crop, and is a leading source of calories and protein in the human diet. Better understanding of the wheat genome will enhance future breeding efforts to perpetuate its critical role in feeding the world. Consistent with its superlative nature, wheat also represents the most challenging plant genome to be attempted to date by scientists. At 16,000 million base pairs, its nuclear genome is five times the size of human, eight times the size of maize, and 40 times the size of rice. One explanation for its imposing size is that wheat is hexaploid, i.e., it contains not one but three genomes, termed A, B, and D, that were brought together through hybridization of closely related diploid species during domestication. Because the three genomes are actually quite closely

related, it is difficult to assign genes to their appropriate genome. Making matters more complex, the wheat genome is also one of the most repetitive, with ~90% of its content bloated with transposable elements that obscure the discovery of functioning genes.

These facts have necessitated multiple international initiatives to sequence wheat, overseen by the International Wheat Genome Sequencing Consortium (IWGSC), wherein individual chromosomes have been divvied up for work by individual labs. Our lab is a collaborator on the National Science Foundation (NSF)-funded project to physically map chromosomes 1D, 4D, and 6D of bread wheat (cultivar Chinese Spring) and all seven chromosomes of the closely related species *Aegilops tauschii*. This latter species is highly informative because it is the diploid progenitor of the wheat D genome. Nearing completion, the project has produced physical maps containing almost three-quarters of a million bacterial artificial chromosomes (BACs), positioned relative to one another by a restriction enzyme fingerprinting method. The project has also produced the first high-resolution genetic map of *Ae. tauschii*, with more than 7000 sequenced markers that are anchored to the physical map. These maps are scheduled for public release this year via the Gramene web portal. Gramene will also display comparative maps between the D genome and other grass reference genomes to enable study of gene function and evolution. Together, the information and physical reagents produced by this project will help lead the way for future research in wheat genomics.

Funded by a second NSF grant, CSHL is pioneering the discovery of wheat genes by using ultra-high-throughput sequencing technologies and rapidly releasing these data to the scientific community. In this whole-genome shotgun approach, enormous quantities of sequence data can be generated in a cost-effective manner. To date, the McCombie lab has deposited more than 850 Gb (~200-fold coverage) of *Ae. tauschii* to the NCBI Sequence Read Archive (SRA). Similar numbers have been deposited for *Triticum monococcum*, the diploid progenitor of the wheat AA genome. Efforts are under way in our lab to assemble these sequences and build scaffolds to describe the gene content of these genomes. Using physical/genetic maps such as those described above, and by comparative mapping to other grass species, we plan to position many of the genes to their location in the genome. Results will be released via a browser within Gramene. Work by CSHL complements extensive research efforts taking place worldwide that together will ultimately tackle the wheat genome.

The Maize Methylome

Z. Lu, A. Olsen, S. Pasternak [in collaboration with R. Martienssen, J. Hicks, W.R. McCombie, M. Regulski, and J. Kendall, Cold Spring Harbor Laboratory; S. Tingey and A. Rafalski, Pioneer/DuPont]

DNA methylation has an important role in the regulation of gene expression and control of transposable elements. The patterns of DNA methylation, referred to as the methylome, must be faithfully propagated for proper development in plants and animals. We are collaborating with the Martienssen lab and DuPont to sequence the methylome of two maize inbred lines, B73 and Mo17 (Fig. 1). The genomic DNA is treated with bisulfite, which will convert unmethylated cytosine to thymine. Sequencing using next-generation Illumina GA2 paired-end reads, followed by mapping back to the maize genome, resulted in identification of a methylome in single-base resolution. We have generated 20×–30× coverage over the mappable portion of the maize genome. Alignment with RNA sequences indicates that the methylation patterns are correlated with gene expression, small RNA, and alternate splicing. Diversity in cytosine methylation patterns was observed in transposable elements and especially in genes and was found

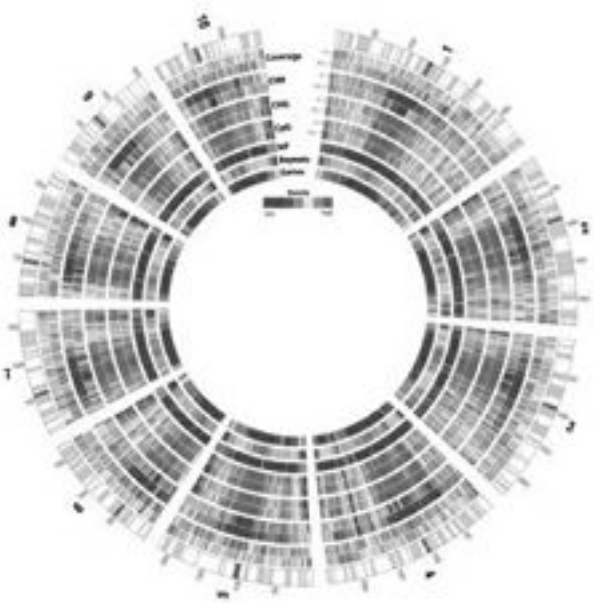

Figure 1. Distribution of methylated sites within the maize nuclear genome. Feature density is given in 1-Mb bins. (Coverage tracks) Average read depth, (CpG/CHG/CHH tracks) average methylation level, (MF track) methylation filtration density, (repeats track) repeat density, (gene track) gene density.

to be largely heritable in recombinant inbred lines (RILs). However, significant deviations from heritability were observed, many of which were conserved in different RILs. This will help us to reveal the roles of DNA methylation in gene regulation and other biological functions in the future.

Maize HapMap

J.-M. Chia [in collaboration with E. Buckler, USDA, Cornell University; M. McMullen, USDA, University of Missouri, Columbia]

Maize breeders have long exploited the diversity in maize for improving its yield through selective breeding programs, contributing to its current position at the apex of the production crop list. We are in an era in which traditional crop-breeding techniques are being supplemented, and in some cases supplanted, by molecular breeding approaches that speed up the breeding process. Understanding the diversity in maize and how it impacts agronomic traits is crucial in implementing these new approaches and will be an important platform for sustainable agriculture in the future.

In the previous year, we sequenced more than 100 inbred lines of the *Zea mays* genus. The volume of sequences generated per inbred line is approximately five times the size of the genome, and from these sequences, we can identify variant loci. Given the complexity of the genome, these variations were scored using carefully designed population-genetics-based filters that leverage the carefully constructed maize breeding populations. In total, more than 55 million SNPs were identified.

We are currently extending our sampling across diverse maize landraces with a target of an additional 40 samples, including 15 at high depth (20×). This will allow us to assemble these genomes and identify novel genes that are not present in the elite breeding lines used commercially.

Tracking the Wild Tomato

J.-M. Chia, S. Pasternak [in collaboration with Z. Lippman, Cold Spring Harbor Laboratory]

This work also contributed to the Tomato Genome Sequencing Project (principal investigator, G. Giuliano, ENEA CR Casaccia, Italy), which included multiple coprincipal investigators and researchers from the United States and internationally.

To explore variation between tomato and its nearest red-fruited relative, together with the Lippman lab, we sequenced, analyzed, and assembled a *Solanum pimpinellifolium* genome using Illumina short reads. We focused on identifying SNP and insertion/deletion (InDel) variation by aligning the quality-trimmed reads to the pseudomolecule reference of the cultivated tomato, *S. lycopersicum* cv. Heinz. This revealed surprisingly low genome-wide diversity, supporting an extremely close evolutionary relationship with Heinz that is consistent with *S. pimpinellifolium* serving as the foundation for tomato domestication. Assembling the reads into sequence contigs, we did not see any large structural variations between the two genomes. This extensive molecular marker database that is now available makes it possible to access *S. pimpinellifolium* variation for crop improvement. In particular, the diversity in the undomesticated species for adaptation and disease resistance will be particularly valuable.

The iPlant Collaborative

Z. Lu, C. Noutsos, S. Pasternak, J. Stein

This project employs more than 100 staff and is headquartered at the University of Arizona (principal investigator is Stephen Goff). Dozens of collaborators are located at more than 20 institutions.

Driven by the large-scale generation of data, ranging from genomics to imaging, research in biology is becoming increasingly information based. Lagging behind is the ability of individuals to make efficient use of this information due to limitations in data access and storage, computational infrastructure, and available tools. To tackle big questions in biology, researchers also need effective ways to collaborate that reach across institutional and disciplinary lines. The iPlant Collaborative (http://iplantcollaborative.org) is an NSF-funded cyberinfrastructure project that provides public access to high-performance computing, data storage, and tools via customized web-based interfaces. Having completed year 4 of this 5-year grant, the iPlant Collaborative has made extensive progress toward meeting these goals. Work in the last year has culminated in the development of multiple platforms that are now being used by the research and educational communities. Staff at CSHL directly contributed to some of these cyberinfrastructure platforms or have built upon them to provide ready access to needed software and analysis tools by plant scientists and educators. Within our lab, these platforms include the

Discovery Environment (DE), Atmosphere, and Taxonomic Name Resolution Service (TNRS).

The DE is perhaps the most visible portal to iPlant tools and services. This web-based platform supports an "app store" model of user-extensible tools, workflows, and data storage. Users can take advantage of existing tools contributed by the iPlant staff and user community or develop their own tools to use privately or share. Although users may not be aware, the underlying infrastructure provides access to iPlant's massive data store, and in the case of computer-intensive tasks are using advanced computing services such as the Extreme Science and Engineering Discovery Environment (XSEDE) through the Texas Advanced Computing Center (TACC). So far, well over 100 tools have been integrated into the DE that enable a broad range of research activities, including genome/transcriptome assembly, RNA-Seq quantification, variant detection, GWAS, and phylogenetics. Members of our lab have had important roles in contributing to workflow design, tool integration, validation, and documentation.

Atmosphere is iPlant's cloud infrastructure platform that addresses the growing need for configurable and cloud-enabled computational resources by the plant research community. From Atmosphere's web interface, users can launch a virtual machine (VM) with preconfigured working environments and ready-to-use software. Users can also create their own applications and environments and post their VM in Atmosphere for others to use. As with the DE, Atmosphere is a gateway to access iPlant's core infrastructure resources, such as the high-performance and grid-computing environment and big data storage system. Using the Atmosphere platform, we created a VM to be used in the fields of ecological and functional genomics. The VM includes 19 R statistics packages used in ecology and genetics research and for plotting complex data in graphs.

Our lab contributed directly to the development of the Taxonomic Name Resolution Service (TNRS), a platform to help standardize taxonomic names for all plant species—a nontrivial task. Erroneous and synonymous taxonomic names are a major challenge for virtually every field of plant biology. Large organismal databases (GBIF, SpeciesLink, VegBank, SALVIAS, TraitNet, GenBank, TreeBASE) are plagued by taxonomic error and uncertainty. In some databases, up to 30% of names do not match to any published name. Furthermore, 5%–20% of published names may be synonymous. Currently, correcting and harmonizing taxonomy is the time-consuming and ad hoc responsibility of the individual researcher. The TNRS tool overcomes this barrier, enabling higher-quality comparative biodiversity science. TNRS is available to other investigators who wish to perform similar taxonomic name resolutions on their data sets, enabling a wider community to expand the public scientific knowledge base. The TNRS is currently available at http://tnrs.iplantcollaborative.org.

A major mission of iPlant is to promote adoption of the cyberinfrastructure through training workshops and outreach. In November 2011, members of our lab and the DNA Learning Center organized the iPlant Tools and Services Workshop at CSHL to coincide with the Plant Genomes and Biotechnology meeting. This 1-day workshop focused on training in the Discovery Environment for RNA transcript abundance and phylogenetics analysis and touched on additional topics such as genome-wide association study (GWAS), Atmosphere, and the iPlant Data Store. The workshop was attended by nearly 40 lab heads, research staff, postdocs, and graduate students hailing from institutions around the world, including Argentina, Germany, India, South Korea, and Singapore. The workshop was a success not only in terms of training, but also in receiving valuable feedback that will further strengthen iPlant in the future.

Plant Systems Biology: Exploration of Gene Regulatory Network Guiding *Arabidopsis* Stele miRNA Expression

L. Zhang, S. Kumari, Y.K. Lee [in collaboration with S. Brady, University of California, Davis]

The gene regulatory network (GRN), comprising both transcriptional and posttranscriptional pathways, governs many processes of development and response to environmental stress. A GRN is composed of several types of interactions that regulate gene expression, including protein–DNA, protein–protein, and microRNA (miRNA)–mRNA interactions. To map protein–DNA interactions, a gene-centered yeast one-hybrid (Y1H) method enables systematic and comprehensive identification of upstream-bound transcription factors (TFs) that regulate expression of certain genes and miRNAs. To characterize TF interactions in the root stele, we first developed a resource in *Arabidopsis* based on the Y1H method. It consists of 653 full-length TFs, which represent 92% of root stele-expressed TFs and 74.5% of root-expressed TFs (Fig. 2). We also improved our screening protocol to replace the labor-intensive direct transformation method with the more rapid mating-based assay and also used a high-copy

activation domain vector. The process has been automated with robotics and includes four technical replicates (Brady et al. 2011). We tested our resource using 13 stele-expressed TF promoters, which revealed 158 interactions, many of which have been demonstrated to occur physically in planta or are associated with altered molecular phenotypes in response to genetic perturbation.

Using this resource, we have focused our effort to map a comprehensive GRN that integrates miRNA-based posttranscriptional regulation with TF-based transcriptional regulation within root stele in *Arabidopsis.* Approximately 60 miRNA promoters have been screened together with promoters from portions of miRNA targets and promoters of highly connected TFs. To validate in vivo the protein and DNA interactions that resulted from the Y1H data set, we recently characterized homozygous knockout lines in *Arabidopsis* for 74 candidate TFs by quantitative polymerase chain reaction (Q-PCR) and 20 TFs by Taqman miRNA assays. Here, we characterized molecular expression phenotypes for TFs and their miRNA targets. These data showed that plants mutant in ~80% of the TFs and 70% of the miRNAs tested display molecular phenotypes.

We continue to use this resource to investigate key regulatory interactions specific to the *Arabidopsis* root stele, as well as how this GRN overlaps with other regulatory networks. Additionally, we are extending our findings to maize in cross-species comparisons and developing an analogous Y1H resource for maize based on orthologous TFs and conserved promoter sequences. Therefore, this work represents a useful resource for elucidating gene regulatory mechanisms at the tissue-specific level with strong potential for translation to important crop species.

Characterization of miRNAs in Response to Short-Term Waterlogging in Three Inbred Lines of *Zea mays*

Z. Liu, S. Kumari [in collaboration with Y. Zheng, Huazhong Agricultural University, China]

Waterlogging of plants leads to low oxygen levels (hypoxia) in the roots and causes a metabolic switch from aerobic respiration to anaerobic fermentation that results in rapid changes in gene transcription and protein synthesis. Our research seeks to characterize the gene regulatory networks associated with short-term waterlogging. In addition to characterizing the involvement of miRNAs and their targets in response to short-term hypoxia conditions, a quantitative reverse transcriptase–PCR (qRT-PCR) assay was used to quantify the expres-

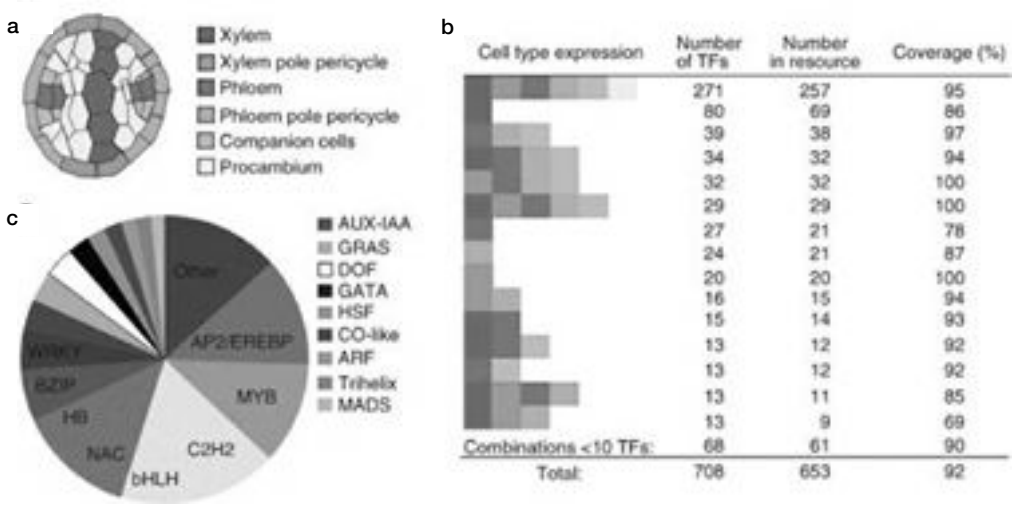

Figure 2. An *Arabidopsis* eY1H transcription factor resource. (*a*) Schematic of root stele cell types. (*b*) Stele cell-type expression profiles of the transcription factors present in our collection. Shading is as in *a*. (*c*) Family categorization of transcription factors within the resource. "Other" represents families with fewer than eight members. (GRAS) Gibberellic acid–insensitive, repressor of GA1 and scarecrow, (DOF) DNA binding with one finger, (GATA) GATA DNA motif, (HSF) heat shock factor, (CO-like) Constans-like, (ARF) auxin response factor, (MADS) MCM1, Agamous, Deficiens, and SRF box, (HB) homeobox, (BZIP) basic leucine zipper. (From Gaudinier et al. 2011.)

sion of the 22 candidate mature miRNA signatures (representing 64 miRNA loci) and their 84 predicted targets in three inbred *Zea mays* lines (Mo17, B73, Hz32) that showed different tolerance to waterlogging. Study results suggest that miR159, miR164, miR167, miR393, miR408, and miR528, already known to have roles in root development and stress responses, also have roles in regulating responses to short-term waterlogging conditions. Furthermore, computational approaches were used to predict the stress- and development-related *cis*-regulatory elements within the promoters of these miRNAs using the B73 reference genome and to construct a probable miRNA-mediated gene regulatory network in response to short-term waterlogging stress. The differential expression patterns of miRNAs and their targets in these three inbred lines suggest that candidate miRNAs are active participants in signal transduction at the early stage of hypoxia conditions via a gene regulatory network. We also hypothesize that cross-talk takes place between different biochemical pathways.

Developmental Networks Controlling Inflorescence Architecture in Maize

A. Eveland [in collaboration with D. Jackson, Cold Spring Harbor Laboratory]

The goal of this work is to integrate genetics and genomics data sets to elucidate molecular networks that determine the morphology (architecture) of maize inflorescences. Because inflorescences bear the fruits and grains that we eat, understanding the genetic and regulatory basis for how these structures are formed has clear relevance to important agronomic traits such as grain yield and harvesting ability. Our data sets represent maize inflorescence primordia sampled during key developmental transitions and in perturbed genetic backgrounds. The latter includes loss-of-function mutants in three important regulators of the *RAMOSA* (*RA*) pathway, which controls stem-cell-fate decisions and ultimately the decision to branch. We have established a robust system to investigate the networks that modulate branching, including characterization of the precise timing of developmental events and associated spatiotemporal changes in gene expression. We integrated genome-wide mRNA-Seq data to resolve coexpression networks during key stages of maize inflorescence development, and we are working to expand these networks by incorporating additional data sets, such as genome-wide TF occupancy profiles and *cis*-regulatory information.

Primary objectives for this project completed in 2011 were as follows: (1) Establish a comprehensive pipeline for mRNA-Seq and chromatin immunoprecipitation (ChIP)-Seq data analysis in maize by evaluating and optimizing available software for mapping and quantification, as well as testing various statistical methods to extract biological relevance. (2) Characterize genome-wide expression signatures specific to a given developmental event or branching phenotype. We made use of known developmental marker genes and their spatiotemporal transcriptional responses to genetic perturbation in order to test our experimental system and establish a proxy for developmental staging. (3) Evaluate and implement clustering approaches to identify candidate genes that are coexpressed with key regulators and/or coincide with specific developmental events. Our results included identification of candidate genes, specifically developmentally regulated TFs, and novel genes of unknown function, which are potentially involved in stem cell maintenance and determinacy. We continue to interrogate coexpression clusters for enrichment of functional processes and *cis*-regulatory motifs that are proximal to the transcriptional start site of coexpressed genes (with Sunita Kumari). (4) Identify targets of the *RA1* TF using ChIP-Seq and integrate results with data from parallel mRNA-Seq experiments. On the basis of this approach, we showed that one-third of the genes with altered expression levels in the ra1 mutant are also bound by *RA1*. We are incorporating additional ChIP-Seq data sets as they become available to investigate combinatorial binding of TFs associated with the branching pathway. The ChIP-Seq data also provide in vivo confirmation for binding sites of developmental regulators in maize, information that is being leveraged in efforts to resolve *cis*-regulatory modules across the maize genome.

In the next phase, we will further prioritize candidates from this work by overlaying the *Arabidopsis* regulatory network information (see previous section). Resulting hypotheses can be tested in *Arabidopsis* (e.g., responses to stress) and ultimately translated to agronomic systems. Additionally, candidate genes that are maize- and/or grass-specific are of high priority because they may contribute to the unique morphology of maize inflorescences and/or features shared among other grasses. We will further use comparative genomics approaches, including both computational methods and integration of analogous RNA-Seq data sets from closely related grasses such as sorghum, to identify candidate genes that may contribute to grass-specific aspects of inflorescence architecture.

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Doreen Ware (*bottom, second from left*) and lab members

QUANTITATIVE BIOLOGY

Gurinder S. "Mickey" Atwal and colleagues are applying insights from the physical and computational sciences to the study of population genetics and human disease. In collaborations with colleagues at the Institute for Advanced Study in Princeton, Atwal has modeled the process by which genetic variants, or alleles, have evolved in the last 100,000 years of human history. This has recently led to surprising insights about the role in female fertility played by *p53*, a master tumor suppressor gene, and furthered our understanding of how complex gene networks evolve. Recently, Atwal has analyzed the physical organization of the cancer genome and its role in mediating tumorigenesis across numerous tissue types.

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 honed 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 they 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, Krasnitz and colleagues seek to learn how specific cancer types evolve and how cancer cells migrate in metastasis.

Michael Schatz is a computational biologist and an expert on large-scale computational examination of DNA sequencing data, including the alignment, assembly, and analysis of next-generation sequencing reads. These methods have been used to reconstruct the genomes of previously unsequenced organisms, to probe sequence variations, and to explore a host of biological features across the tree of life. Recent improvements in sequencing technologies are challenging our capacity to store and analyze the huge volume of DNA sequence data being generated. In view of this, Schatz is particularly interested in capitalizing on the latest advances in distributed and parallel computing, especially cloud computing technologies, to advance the state of the art in bioinformatics and genomics.

QUANTITATIVE BIOLOGY

G.S. Atwal R. Aboukhalil C. Liu
Y. Cai Y. Mo
B. Fendler S. Shringapure
W. Liao

Fueled by data generated from recent technological developments in DNA sequencing, our lab is currently focused on population genetics, cancer biology, and high-performance computing. We often tackle scientific questions computationally by invoking theoretical concepts from statistical physics and machine learning.

Suyash Shringapure, a graduate student in the computer science department at Carnegie Mellon University, continues to collaborate with us and Bud Mishra (New York University). Ying Cai is a graduate student from the Applied Mathematics and Statistics department at Stony Brook University and Bernard Fendler is a postdoctoral associate with training in theoretical physics. Willey Liao and Yifan Mo are graduate students from Michael Zhang's group and have continued to work with him since his departure to the University of Texas at Dallas. Chengyu Liu was an URP student during the summer of 2011. Robert Aboukhalil worked for a week in the summer before entering the Watson School as a graduate student.

Identification of *PHLPP1* as a Tumor Suppressor Reveals the Role of Feedback Activation in *PTEN*-Mutant Prostate Cancer Progression

In collaboration with the Trotman Lab at CSHL, we investigated the synergistic co-deletion of PTEN, TP53, PHLPP1, and PHLPP2 in metastatic human cancer samples. Specifically, we analyzed the gene copy-number profiles of patient data and carried out a genome-wide analysis of the pairwise mutual information between gene mutational events. A false discovery rate analysis, based on an adaptive Monte Carlo statistical testing procedure, demonstrated that PTEN and PHLPP1 were significantly co-deleted in metastatic samples and tightly correlated to deletion of TP53 and PHLPP2. These data establish a conceptual framework for progression of PTEN-mutant prostate cancer to life-threatening disease.

Regulation of Human Fertility by Single-Nucleotide Polymorphisms in the P53 Pathway

We continued our work in collaboration with Cornell Medical School and the Cancer Institute of New Jersey, investigating the association of human fertility and genetic polymorphisms 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 single-nucleotide polymorphisms (SNPs) in the p53 pathway that can modify the function of p53 were assembled and used to study their impact on human fertility. Selected alleles in SNPs in *LIF, Mdm2, Mdm4*, and *Hausp* genes, each of which regulates p53 levels in cells, were found to be enriched in IVF (in vitro fertilization) patients. More recently, we have begun to investigate the association of SNPs in p63 and p73. Initial results are encouraging, although we await an increase in sample numbers before the results can be deemed to be statistically significant.

Localization of Tumor Suppressor Genes

In collaboration with the Lowe Lab (now at Memorial Sloan-Kettering Cancer Center), we have carried out a systematic analysis of the spatial distribution of known tumor suppressor genes (TSGs) to test the hypothesis that there exists an evolutionary selection pressure to cluster TSGs throughout the genome. To this end, we developed a computational and analytical model to determine the extent to which we expect to find adjacent TSGs under no selection. Preliminary results show a strong tendency for TSGs to cluster, and this clustering occurs across many species. The conservation of

TSG clusters across species, despite significant chromosomal rearrangements between species, suggests natural selection has acted to preserve the relative TSG order. Ongoing investigations are focused on understanding the implications of this spatial distribution of TSGs for the risk and development of human cancer. Furthermore, we are now investigating the spatial distribution of known oncogenes.

Cell-Type-Specific Spatiotemporal Gene Expression Dynamics Reflects Cerebellar GABAergic Circuit Development

In collaboration with the Huang Lab at CSHL, we have analyzed the temporal gene-expression dynamics of two GABAergic neuron subtypes in developing mice. The objective was to unravel the cell-type-specific molecular correlates of a developing circuit, in contradistinction to the prevailing wisdom that the expression landscape remains relatively static throughout postnatal development. We developed methods based on a random matrix theory to determine the significance of principal component analyses of temporal expression profiles. A number of distinct temporal patterns, from day 0 to day 56, were found for a large subset of genes corresponding to distinct biological pathways. In addition, the research (1) uncovered groups of novel cell-type-specific genes, (2) captured the phasic expression dynamics of ion channels, receptors, cell adhesion molecules, gap junction proteins, and transcription factors, (3) revealed the active developmental modulation of different molecular pathways involved in circuit formation, and (4) showed evidence of hundreds of genomically clustered and developmentally coexpressed transcripts, suggesting the involvement of chromatin regulatory forces in early postnatal development as well as maintenance of cell-type identity in the adult stages.

Recent Coselection in Human Populations

In collaboration with Suyash Shringapure from Carnegie Mellon University and Bud Mishra from New York University, we have devised a method of detecting recent molecular coselection where the selected alleles have not yet reached fixation. A variety of selective forces, including diverse habitats and diseases, have been acting on human populations since their earliest migration out of Africa. Studying recent natural selection through statistical analysis of human genetic polymorphisms is therefore an important way of illuminating this recent human history. In the last few years, a number of methods have been proposed for detecting recent natural selection. These methods have found a large number of genes in human populations showing strong signals of recent natural selection, but they have not been able to find groups of genes (not necessarily syntenic) that might have responded to shared selective forces. For instance, because many of these genes are involved in various common biological pathways and networks, we hypothesize that selection pressure on one gene in a network will result in a simultaneous selection pressure on other genes in the network. We have analyzed SNP data from the HapMap project to find candidates for recent coselection in human populations.

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IN SILICO CANCER GENOMICS

A. Krasnitz V. Ignatova
M. Kato
G. Sun

Research in our group is focused on in silico cancer genomics. In the last 5 years, there has been explosive growth in the volume as well as quality and detail of cancer-related genomic data available for analysis. This development came about through rapid accumulation of public data sets generated by individual laboratories worldwide, through large-scale cancer genome projects such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), and with the advent of new experimental methodologies, especially the next-generation sequencing. We see our goal in channeling this flood of data into a number of clinically relevant applications, including pinpointing and prioritizing targets for functional analysis, discovery of genomic markers for clinical outcome, and molecular classification of cancer. All our work is done in close coordination with experimental studies performed by M. Wigler, J. Hicks, S. Powers, S. Lowe (presently at Memorial Sloan-Kettering Cancer Center), and B. Stillman laboratories at CSHL.

Recurrence in Cancer Genomes

Large collections of intervals are a common form of data in high-throughput genomics. In the context of chromatin immunoprecipitation–high-throughput sequencing (ChIP-Seq) or cross-linking immunoprecipitation–high-throughput sequencing (CLIP-Seq) analyses, these intervals represent fragments of DNA or RNA. DNA copy-number analysis yields intervals of the genome corresponding to gains or losses of copy number. In all cases, a common goal is inference of contiguous genomic regions covered by the intervals with a high degree of recurrence. We call such regions cores. In cancer, genomes display complex patterns of DNA copy-number alteration, but recurrent aberrations are observed within a given cancer type. Detection and quantitative characterization of the cores in a given malignancy are potentially beneficial in two ways. First, it is plausible that at least some of the recurrence owes to selective pressure on regions harboring cancer-related genes. Focusing on these regions may therefore facilitate cancer gene discovery. Second, once the cores are known, the genome of a tumor can be described in a drastically simplified fashion by indicating the presence or absence of copy-number alteration in any given core. This simplified form of the data is better suited for further analysis, such as finding subtypes in a given type of cancer or discovering associations between genomic properties and clinical parameters.

We designed and implemented a method for identifying cores in large surveys of genomic interval data. A descriptive name for the method is explanation of recurrent intervals by cores (ERIC). Central to ERIC is the notion of explanatory power. We say that a core explains an event and quantify the explanation provided as a number between 0 and 1. The explanation is a measure of how closely the event is matched by the core. We then seek a set of cores that jointly provide the best possible explanation of the data, subject to additional criteria of statistical significance and robustness under resampling of the input.

In the course of 2011, we used ERIC to process a number of massive collections of cancer genomes. In application to two sets of genomic profiles derived from frozen breast tumor tissues, ERIC revealed cores that are significantly correlated with key histological and clinical parameters, including tumor grade, cell ploidy, presence of *TP53* mutations, and expression-based molecular subtype. Armed with this experience, we applied ERIC to a more challenging set of genomic profiles, generated by Dr. James Hicks at CSHL from formalin-fixed breast tumor tissues of patients with advanced breast cancer who were in two CALGB (cancer and leukemia group B) clinical trials. In this case, we also found highly significant associations between cores and the molecular subtypes. In particular, a core corresponding to amplification of a narrow genomic region harboring the *ERBB2* gene is strongly associated with the *ERBB2* subtype. We also detected cores associated with elevated proliferation of tumor cells. These findings are evidence of a measur-

able impact of such genomic alterations on the disease phenotype. Furthermore, our analysis suggests prognostic utility of the cores. Although trastuzumab is routinely prescribed to patients with an amplification of the *ERBB2* gene or with overabundance of its protein product, we identified two cores as potential markers for benefit from trastuzumab in patients who do not meet these criteria. This finding suggests the possibility of significantly improving the overall survival in ~15% of advanced breast cancer cases.

Genomic Subtypes of Ovarian Cancer

We used ERIC to analyze a massive (516 profiles) high-resolution (Agilent 1.1 million probe CGH platform) DNA copy-number data set accumulated by TCGA for serous ovarian cancer and used the resulting set of cores for genomic subtyping of the disease. With the help of a statistically supported hierarchical clustering technique developed in our lab, we partitioned the set of tumors into four subtypes (a–d) exhibiting strikingly different patterns of copy-number alterations (CNAs). In particular, CNAs are relatively infrequent in subtype a. In contrast, copy-number gains are extremely frequent and present across the entire genome in subtype b. Importantly, the four subtypes differ in additional genomic and epigenomic properties that are not directly related to DNA copy number. Examining sequence-based exon somatic mutation data, we found that somatic point mutation counts per tumor differ significantly ($p = 0.0005$) from subtype to subtype. Next, we compared the subtypes in terms of DNA methylation. Genome-wide microarray-based methylation profiles were available from TCGA for all tumors. We detected significant differences among the subtypes in the degree of methylation for a number of chromosomes, but most dramatically ($p = 2.8 \times 10^{-10}$) for chromosome X.

Furthermore, we observed significant phenotypic differences between the subtypes. Performing multiclass gene set analysis, we found that 26 KEGG pathways are differentially regulated among the subtypes ($q < 0.05$). Most of these pathways fall into functional categories related to cell cycle, DNA-damage repair, immune response, and apoptosis-inducing signaling. Moreover, nearly 100 genes in differentially affected pathways were also differentially expressed individually among the subtypes. This property holds for a number of known key cancer genes whose differential expression patterns help shed light on the phenotypic differences between the subtypes. We consistently find that genes with known oncogenic properties are expressed at elevated levels in subtype b, whereas genes with tumor suppressive properties exhibit reduced expression in that subtype. An inverse pattern is observed for subtype a.

Taken together, these findings suggest that subtype b is associated with a more aggressive form of the disease. We find further support for this expectation when we consider the subtype effect on patient survival. Clinical prognosis is significantly more adverse for patients with subtype-b tumors: Their median overall survival and disease-free survival was shorter by 11 months and 6.5 months, respectively, than those of the remainder of the cohort. A similar shortening in disease-free survival time (by 7.5 mo) is found for patients in the narrower category of complete responders to the initial combined surgical and chemotherapeutic treatment.

Evolutionary Analysis of Single-Cell Sequencing Data in Breast Cancer

Single-cell sequencing is a promising technology for revealing properties of single cells that are missed by sequencing DNA from macroscopic amounts of tissue. A recent study by the Wigler lab at CSHL using this technology accurately determined DNA copy-number profiles of 100 cells in breast cancer tissue of an individual. Subsequent phylogenetic analysis identified several distinct subpopulations of cells and pointed to a possibility of punctuated evolution profiles.

To further characterize cancer cell evolution, we analyzed the 100-cell sequencing data in the framework of population genetics. In this framework, we viewed cancer cells as individuals of a nonsexual species and DNA copy-number losses as a form of mutation. Applying this concept, we found that most losses were selectively neutral but that some were exposed to natural selection. Tumor suppressor genes appear to be involved not only in positive selection, but also in balancing selection. We also estimated population genetic parameters such as growth rates and mutation rates from the combination of the copy-number data and data from flow cytometry. Our framework will shed light on cancer as an evolutionary process and will help to screen for genomic regions that are important for cancer cells from the viewpoint of evolution.

COMPUTATIONAL SEQUENCE ANALYSIS

M. Schatz J. Gurtowski G. Narzisi
H. Lee M. Titmus

Our lab develops novel computational analysis methods to study the information content in biological sequences and the dynamics of biological processes. These include methods for assembling next-generation sequence data into complete genomes, aligning sequences to discover variations or measure transcription levels, and mining these data to discover relationships between an organism's genome and its traits. We then apply these methods to study several biological systems, for example, to discover mutations associated with human diseases such as cancer and autism or to reconstruct the genomes of important agricultural crops and biofuels.

In the past year, our lab has grown by several new members. Hayan Lee joined in January, and over the summer, she has become a candidate in the computer science Ph.D. program at Stony Brook University. She is developing novel machine-learning algorithms to study the limits of variation detection and to improve genome-wide association studies. During the summer, Paul Baranay visited the lab from the University of Notre Dame in the CSHL undergraduate research program. He is developing new computational methods for improving genome assembly, which he applied to the assembly of the cichlid fish in the international Assemblathon-2 competition. In November, James Gurtowski and Giuseppe Narzisi joined our lab as informatics developers. James was previously an analyst at the Mount Sinai School of Medicine, and he is now working on developing analysis tools for optimizing the growth of plants as biofuels. Giuseppe recently completed his Ph.D. in computer science at New York University and is now developing novel computational methods for studying the molecular basis of autism.

CSHL Adjunct Associate Professor D. Ware and I were awarded a 5-year, $5 million grant from the Department of Energy to study and optimize the growth of plants as biofuels through the development of a Knowledgebase in Systems Biology. I have also formalized this collaboration with CSHL Professor M. Wigler to continue studying the molecular basis of autism, and with Johns Hopkins University Professor S. Salzberg to develop large-scale computational sequence analysis methods for human and plant genomics. In May, I was awarded the CSHL Winship Herr Award for Excellence in Teaching for contributions to the new CSHL course in Quantitative Biology, co-taught by M. Atwal. In the last year, I have also been interviewed by editors of the journal *Science*, the trade publication *Genome Technology*, *Reuters*, and *The New York Times*, especially to describe my work with applying cloud-computing technologies to help address the data deluge in genomics.

Competitive Genome Assembly and Analysis

A major research focus of our lab is developing enhanced methods for de novo genome assembly, which aims to reconstruct the genome of an organism from billions of short DNA sequences. We apply these methods to reconstruct the genomes of previously unsequenced organisms, including several plant and animal species. Current sequencing technology can now be used to sequence the equivalent of an entire human genome in a matter of days and at low cost. Sequence read lengths, initially very short, have rapidly increased since the technology first appeared, and we now are seeing a growing number of efforts to sequence large genomes de novo from these short reads such as the Genome 10k project that aims to sequence 10,000 vertebrate genomes.

The past year saw two major efforts to benchmark the state of the art in genome assembly. The first such effort, the Assemblathon, was organized by researchers at the University of California, Santa Cruz. In a collaborative effort, teams were asked to assemble a simulated Illumina HiSeq data set of an unknown, simulated diploid genome. A total of 41 assemblies from 17 different groups were received, including two constructed by our laboratory. Novel haplotype aware assessments of coverage, contiguity, structure, base calling, and copy number were made and established that (1) it is possible to assemble the genome to a high level of coverage and accuracy and (2) large differences exist between the assemblies, suggesting room for further improvements in current methods. The simulated benchmark, including the correct answer, the assemblies, and the code that was used to evaluate the assemblies, is now public and freely available.

In a second project called the Genome Assembly Gold Standard Evaluations (GAGE) project, organized by re-

searchers at Johns Hopkins University, we evaluated several of the leading de novo assembly algorithms on four different short-read data sets, all generated by Illumina sequencers. Our results describe the relative performance of the different assemblers as well as other significant differences in assembly difficulty that appear to be inherent in the genomes themselves. Three overarching conclusions are apparent: (1) data quality, rather than the assembler itself, has a dramatic effect on the quality of an assembled genome, (2) the degree of contiguity of an assembly varies enormously among different assemblers and different genomes, and (3) the correctness of an assembly also varies widely and is not well correlated with statistics on contiguity. To enable others to replicate our results, all of our data and methods are freely available, as are all assemblers used in this study.

These two studies were important for benchmarking the state of the art in assembly. However, unlike these studies working from a known reference sequence, it remains an open problem for analyzing an assembly of an unknown genome to identify any errors. Along these lines, members of our lab published a revision to the AMOS project with improved methods for visualizing and assessing the quality of a genome assembly. Since its launch in 2004, the open-source AMOS project has released several innovative DNA sequence analysis applications including Hawkeye, a visual analytics tool for inspecting the structure of genome assemblies; the Assembly Forensics and FRCurve pipelines for systematically evaluating the quality of a genome assembly; and AMOScmp, the first comparative genome assembler. These applications have been used to assemble and analyze dozens of genomes ranging in complexity from simple microbial species through mammalian genomes. Recent efforts have been focused on enhancing support for new data characteristics brought on by second- and now third-generation sequencing. The revised software emphasized the methods for assessing assembly quality and the visual analytics capabilities of Hawkeye. These interactive graphical aspects are essential for navigating and understanding the complexities of a genome assembly, from the overall genome structure down to individual bases. Hawkeye and AMOS are freely available and open-source software.

Ultra-Large-Scale Biological Sequence Analysis

In the race between DNA-sequencing throughput and computer speed, sequencing is winning by a mile. Sequencing throughput is currently about 500–600 billions of bases per run on a single sequencing machine, and it is improving at a rate of about fivefold per year. In comparison, computer performance generally follows "Moore's Law," doubling only every 18 or 24 months. As the gap in performance widens, the question of how to design higher-throughput analysis pipelines becomes crucial. One option is to enhance and refine the algorithms to make better use of a fixed amount of computing power. Unfortunately, algorithmic breakthroughs of this kind, like scientific breakthroughs, are difficult to plan or foresee. The most practical option is to develop methods that make better use of multiple computers and processors in parallel.

Parallel computing systems have existed for decades in different forms, but a system called MapReduce is especially promising for accelerating data-intensive genomics research. MapReduce was developed at Google for their large data analysis, especially for scanning across billions of webpages to find the most relevant pages for a search query. The power of MapReduce is that it can intelligently distribute computation across a cluster with hundreds or thousands of computers, each analyzing a portion of the data set. The system was originally developed for text and web processing at Google, but an open-source implementation called Hadoop is now available to install on any cluster for any application domain.

The first ever application of this technology to genomics (which I published in 2009) was to accelerate the short-read mapping algorithm CloudBurst. In CloudBurst, many computers work together using Hadoop to map reads to a reference genome, allowing for any number of differences to determine from where each read originated and if any variations are present. In experiments with 96 cores, the mapping algorithm was ~100 times faster than using a single core, thus reducing the run time from several weeks to a few hours. The compute cluster used for these experiments was completely virtual and was leased from the Amazon Elastic Compute Cloud (EC2), which offers computing time in their cloud at a small fee per node per hour used.

These pioneering efforts have allowed numerous applications to be developed to leverage Hadoop and cloud computing for genomics. Several of these applications have focused on accelerating sequence alignment, because it is one of the most important applications in computational biology, and it is used for such diverse tasks as identifying homologous proteins, analyzing gene expression, mapping variations between individuals, or assembling de novo the genome of an organism. Except for trivial cases involving just a small number of short sequences, virtually all other sequence alignment tasks rely

on a precomputed index of the sequence to accelerate the alignment. Two of the most important index structures are the suffix array, which consists of the lexicographically sorted list of suffixes of a genome, and the closely related Burrows-Wheeler Transform (BWT), which is a permutation of the genome based on the suffix array. Constructing these structures on large sequences, such as the human genome, requires several hours of serial computation and must be performed for each genome, or genome assembly, to be analyzed. In May, I, along with two master's students whom I was supervising in the computer science department at Stony Brook University, published the first algorithm for constructing the suffix array and the BWT of a sequence leveraging the unique features of MapReduce. We demonstrated the performance of the algorithm by greatly accelerating the suffix array and BWT construction on five significant genomes using as many as 120 cores leased from the EC2, reducing the end-to-end run time from hours to mere minutes.

Rate of Meristem Maturation Determines Inflorescence Architecture in Tomato

This work was done in collaboration with the Lippman lab at CSHL.

Flower production and crop yields are highly influenced by the architectures of inflorescences. In the compound inflorescences of tomato and related nightshades (Solanaceae), new lateral inflorescence branches develop on the flanks of older branches that have terminated in flowers through a program of plant growth known as "sympodial." Variability in the number and organization of sympodial branches produces a remarkable array of inflorescence architectures, but little is known about the mechanisms underlying sympodial growth and branching diversity. One hypothesis is that the rate of termination modulates branching. By performing deep sequencing of transcriptomes, we have captured gene expression dynamics from individual shoot meristems in tomato as they gradually transition from a vegetative state to a terminal flower. Surprisingly, we find thousands of age-dependent expression changes, even when there is little change in meristem morphology. From these data, we reveal that meristem maturation is an extremely gradual process defined molecularly by a "meristem maturation clock." Using hundreds of stage-enriched marker genes that constitute this clock, we show that extreme branching, conditioned by loss of expression of the *COMPOUND INFLORESCENCE* gene, is driven by delaying the maturation of both apical and lateral meristems. In contrast, we find that wild tomato species display a delayed maturation only in apical meristems, which leads to modest branching. Our systems genetics approach reveals that the program for inflorescence branching is initiated surprisingly early during meristem maturation and that evolutionary diversity in inflorescence architecture is modulated by heterochronic shifts in the acquisition of floral fate.

Two New Complete Genome Sequences Offer Insight into Host and Tissue Specificity of Plant Pathogenic *Xanthomonas* spp.

This work was done in collaboration with A. Bogdanove, Iowa State University.

Xanthomonas is a large genus of bacteria that collectively cause disease in more than 300 plant species. The broad host range of the genus contrasts with stringent host and tissue specificity for individual species and pathovars. Whole-genome sequences of *X. campestris* pv. raphani strain 756C and *X. oryzae* pv. *oryzicola* strain BLS256, pathogens that infect the mesophyll tissue of the leading models for plant biology, *Arabidopsis thaliana* and rice, respectively, were determined that provided insight into the genetic determinants of host and tissue specificity. Comparisons were made with the genomes of, respectively, closely related strains that infect the vascular tissue of the same hosts, and across a larger collection of complete *Xanthomonas* genomes. The results suggest a model in which complex sets of adaptations at the level of gene content account for host specificity, as well as subtler adaptations at the level of amino acid or noncoding regulatory nucleotide sequence that determine tissue specificity.

Complex Microbiome Underlying Secondary and Primary Metabolism in the Tunicate–Prochloron Symbiosis

This work was done in collaboration with M. Donia, University of Utah.

The relationship between tunicates and the uncultivated cyanobacterium *Prochloron didemni* has long provided a model symbiosis. *P. didemni* is required for survival of animals such as *Lissoclinum patella* and also makes secondary metabolites of pharmaceutical interest. Here, we

studied the metagenomes, chemistry, and microbiomes of four related *L. patella* tunicate samples from a wide geographical range of the tropical Pacific. The remarkably similar *P. didemni* genomes are the most complex so far assembled from uncultivated organisms.

Although *P. didemni* has not been stably cultivated and comprises a single strain in each sample, a complete set of metabolic genes indicates that the bacteria are likely capable of reproducing outside the host. The sequences reveal notable peculiarities of the photosynthetic apparatus and explain the basis of nutrient exchange underlying the symbiosis. *P. didemni* likely profoundly influences the lipid composition of the animals by synthesizing sterols and an unusual lipid with biofuel potential. In addition, *L. patella* also harbors a great variety of other bacterial groups that contribute nutritional and secondary metabolic products to the symbiosis. These bacteria possess an enormous genetic potential to synthesize new secondary metabolites. For example, an antitumor candidate molecule, patellazole, is not encoded in the genome of *Prochloron* and was linked to other bacteria from the microbiome. This study unveils the complex *L. patella* microbiome and its impact on primary and secondary metabolism, revealing a remarkable versatility in creating and exchanging small molecules.

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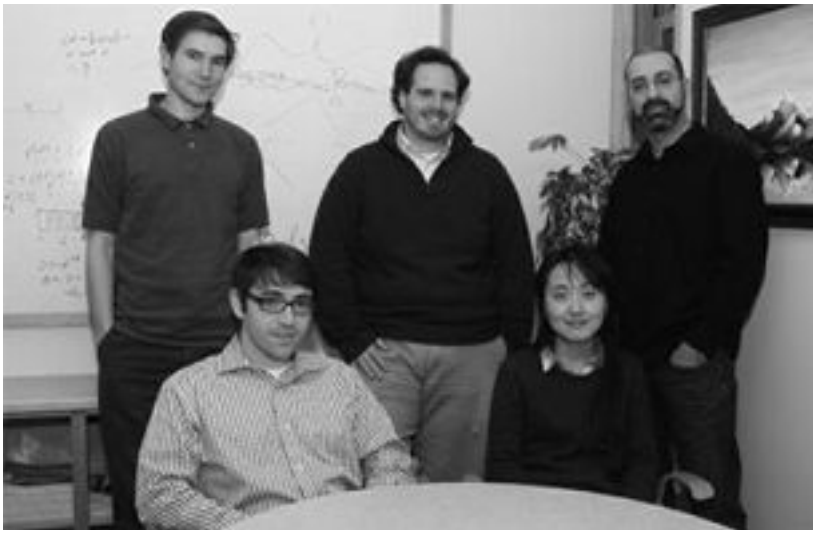

(*Standing*) James Gurtowski, Michael Schatz, Matthew Titmus; (*seated*) Mitchell Bekritsky, Ha Yan Lee

QUANTITATIVE BIOLOGY FELLOWS

Quantitative biology fellows are independent researchers who enter this position immediately after receiving a Ph.D. They come from the fields of mathematics, physics, engineering, or computer science and will spend 3–5 years at CSHL applying these disciplines to biological questions of present and future interest to researchers at the Lab.

Ivan Iossifov focuses on the development of new methods and tools for genomic sequence analysis and for building and using molecular networks, and he applies them to specific biomedical problems. Iossifov studies the genetics of common diseases in humans using two main tools: next-generation sequencing and molecular networks representing functional relationships among genetic loci. These approaches in combination enable the kind of large-scale studies necessary for furthering our understanding of the complex etiology of disorders such as autism, bipolar disorder, and cancer.

Justin Kinney's research combines theory, computation, and experiment in an effort to better understand the relationship between sequence and function in molecular biology. The use of microarray and ultra-high-throughput sequencing technologies has a central role in this work, making possible assays on the activities of tens of thousands to millions of different biological sequences in simple, small-scale experiments. The resulting data can then be used to infer precise quantitative models for how biological sequence dictates function. Kinney has used ChIP-chip and protein-binding microarray data to infer models for the DNA sequence specificities of transcription factors. The same principle can be used to fit models to any data set comprising a list of sequences and corresponding measurements for whatever biological activity in which one is interested. He has also demonstrated that fluorescence-activated cell sorting and 454 pyrosequencing can be used to measure the transcriptional activities resulting from hundreds of thousands of slightly mutated versions of the *Escherichia coli lac* promoter. Measuring the in vivo activity of a large number of slightly mutated regulatory sequences provides a new way of interrogating the transcriptional regulatory code and may be applicable to a wide range of regulatory sequences in a variety of single-celled organisms as well as in cell culture.

Network Analysis of the Genes Affected by De Novo Copy-Number Variants Identified in 1000 Families with One Autistic Child from the Simons Simplex Collection

I. Iossifov

I am studying the genetics of common diseases in humans using two main tools: next-generation sequencing and molecular networks representing functional relationships among genetic loci. In combination, these tools enable large-scale studies necessary for furthering our understanding of the complex etiology of disorders such as autism, bipolar disorder, and cancer. I focus both on the development of new methods—for sequence analysis, for building, and for using molecular networks—and on their applications for specific biomedical problems.

The latest study from Mike Wigler's group at CSHL of the genomic copy-number variation in a large cohort of families with a single child with autism confirmed the role of de novo deletions and duplications in the etiology of autism (Levy et al. 2011). The group identified 75 de novo copy-number events in 68 of the 889 analyzed affected children, whereas only 19 events were observed in 17 of the 895 unaffected siblings. The significantly increased frequency of de novo events argued for their causative role.

The limited recurrence within the 75 events in the autistic children, together with the large number of genes affected by some of the events, led to a list of more than 900 perturbed genes. It is natural to expect that only a small subset of these genes (i.e., one or two per copy-number event) would be related to autism. We assumed that the subset will be composed of genes that are functionally related, and thus, we developed a novel method, network-based analysis of genetic associations (NETBAG) to search for subsets (clusters) of interconnected genes within the context of a functional network (Gilman et al. 2011).

When applied to the 75 copy-number events in autistic children, the NETBAG method did identify a statistically significant gene cluster. The implications of this finding are potentially profound. It complements and strengthens the importance of the de novo copy-number variants identified by Levy et al. (2011), demonstrating the connection between what looked like disparate events. Moreover, the knowledge that the observed diverse genetic alterations affect a compact functional module opens up more concrete avenues for research into early diagnosis: We can analyze genetic variants and judge if they are related to the disease based on the extent to which they perturb the identified cluster. A close inspection of the genes forming the cluster revealed that they were primarily related to synapse development, axon targeting, and neuron motility.

Next-Generation Sequencing for Identifying Genetic Variants

Recent advances in sequencing technology made practical the identification of the genetic variants at a nucleotide level in whole exomes (i.e., all coding exons) of large numbers of individuals. This creates exciting opportunities for studying Mendelian and complex human phenotypes. There are serious problems associated with managing the huge amounts of data, dealing with the substantial levels of noise and biases, and with the interpretation of the identified variants. In collaboration with several groups at CSHL, I tried to address some of these problems by building a whole exome sequence analysis pipeline.

The pipeline is flexible, easy to run, and provides structured internal information that greatly facilitates the interpretation of results. It is almost fully automated, it efficiently utilizes the computational resources at CSHL, it produces detailed statistics that help in monitoring the sequencing process and in optimizing the sample preparation protocols, it handles multiple samples per lane and multiple lanes per sample designs, it produces lists of annotated single-nucleotide polymorphisms (SNPs) and indel variants for every analyzed sample, and it can identify the de novo mutations in families and somatic mutations when given two different tissue samples from the same individual.

The pipeline has been adopted in several projects at CSHL, and two papers whose results were obtained with its help have already been accepted. In collaboration with Jennifer Parla and Dick McCombie, I systematically compared the performance of two leading exome capture kits to that of the whole-genome sequencing approach (Parla et al. 2011). Our analysis indicates that both kits are roughly equal, but stresses some limitations of the exon capture approach, suggesting that with the rapidly decreasing prices, whole-genome sequencing is likely to become the preferred choice. In collaboration with Mona Spector and Scott Lowe, I analyzed the somatic mutations in the whole exome of mast cell leukemia cells from one patient. Among the few identified mutations were an atypical nonsynonymous mutation in the KIT receptor and a nonsynonymous mutation in the β chain of the IgE mast cell receptor (Spector et al. 2011). Had these mutations been known, the course of treatment for the particular patient would have been different.

Ongoing Projects

- **Whole-exome sequencing of the Simons Simplex Collection (SSC).** The SSC is expected to grow to 4000 families, with one child with autism spectrum disorder diagnosis and with at least one unaffected child. Such a collection is ideally suited for identification of de novo mutations with a strong effect on the disorder. The recent improvements of the techniques for enrichment of coding genomic sequence and of the new generation sequencing technology made whole-exome sequencing for a large number of samples feasible. The generated data are of high quality and can be used to identify de novo single-nucleotide, short-indel, and copy-number mutations. This project is being done in collaboration with Michael Wigler and Richard McCombie.
- **Analysis of the overall sharing of the parental genome by concordant and discordant autistic siblings.** The goal is to test the hypothesis that a substantial proportion of the inherited autism is caused by a dominant mutation inherited from the mother, with different mothers having different affected loci. So far, we see a suggestive increase of sharing of the mothers' genome in a cohort of ~300 multiplex families from the AGRE collection with microsatellite markers. The next step is to see if the signal is preserved and hopefully statistically significant in a larger set of 700 families genotyped with a 10K SNP array. This project is being done in collaboration with Michael Wigler and Kenny Ye.
- **Sequencing of treatment-related acute myeloid leukemia (tAML) 5q and 7q chromosomes.** A large proportion of tAML cases present with hemizygous

7q or 5q chromosomes. In collaboration with Mona Spector, we are sequencing 50 samples with the hope of identifying a gene within the 5q and 7q regions with recurrent variants affecting protein sequence.

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Dissecting, Decoding, and Reprogramming Multiprotein–DNA Complexes

J.B. Kinney

Multiprotein–DNA complexes regulate a variety of cellular processes. These large assemblies of protein and DNA act as discrete information processing units: They sense protein concentrations and other cellular signals, perform a computation, and generate an output important for cellular physiology. The DNA that scaffolds each complex contains, within its sequence, instructions for how that complex functions. However, our ability to understand such DNA-encoded regulatory programs, and to reprogram multiprotein–DNA complexes by altering this DNA sequence, remains limited.

Prior to coming to CSHL, I proposed a combined experimental and computational method for dissecting and decoding specific multiprotein–DNA complexes using flow cytometry and ultra-high-throughput DNA sequencing. This method, called Sort-Seq, was first demonstrated in the context of transcriptional regulation with experiments on the *Escherichia coli lac* promoter. Using Sort-Seq, my colleagues and I were able, in living cells, to identify protein binding sites, model the sequence-dependent binding energy of the protein cognate to each site, and even measure the interaction energy between such DNA-bound proteins.

I believe that Sort-Seq and similar techniques have the potential to open up a new area of high-precision, data-intensive biochemistry. My laboratory's efforts are focused on developing both the experimental and computational aspects of these methods and applying them to critical problems in molecular and synthetic biology.

Biophysical Basis of Transcriptional Regulation in *E. coli*

Transcriptional regulation in *E. coli* continues to serve as the primary development platform for Sort-Seq. In particular, the extensive biochemical literature on *E. coli* RNA polymerase and its interactions with transcription factors allows us to interpret Sort-Seq data in this context with much more clarity than in other systems. This work is being pursued in collaboration with Tiberiu Tesileanu (Institute for Advanced Study) and the laboratories of Curtis Callan (Princeton University) and Rob Phillips (California Institute of Technology).

Daniel Jones, a graduate student in the lab of Rob Phillips, visited my laboratory for 2 months in the summer of 2011. During this time, he and I performed Sort-Seq experiments on the *E. coli lac* and *mscL* promoters. Tiberiu Tesileanu and the Callan lab are assisting in the analysis of these data.

The *E. coli lac* promoter is one of the best-studied regulatory systems in all of biology. Still, the quantitative biophysical basis for transcriptional regulation at this promoter remains incompletely understood. Our goal for this system is to build a full biophysical model for how this promoter works in vivo, and to use this system as the primary context in which to investigate the transcriptional regulatory code of *E. coli* more generally. In contrast, the functional structure of the *mscL* promoter has, to our knowledge, never been studied. We are therefore using the *mscL* promoter to test the ability of Sort-Seq to decipher the biophysical basis of a transcriptional regulatory system de novo.

High-Resolution Characterization of DNA Replication Origins in Budding Yeast

My laboratory is also using Sort-Seq-like experiments to elucidate the fine structure of DNA replication origins in the yeast *Saccharomyces cerevisiae*. This work is being pursued in collaboration with the laboratories of Bruce Stillman, Mike Schatz, and Leemor Joshua-Tor (CSHL).

In the summer of 2011, I performed preliminary experiments on two well-studied yeast replication origins, ARS1 and HMR-E. An initial analysis of these

data has already revealed new information about poorly understood sequence elements within these origins. Hayan Lee, a graduate student in the Schatz lab, is assisting in the analysis of these data. Ante Tocilj, a postdoc in the Joshua-Tor lab, is using these results to aid structural studies and is also helping to apply Sort-Seq-like techniques in vitro to study prereplication complex assembly.

Rapid Dissection and Model-Based Optimization of Inducible Enhancers in Human Cells

I am also engaged in an exciting collaboration with Tarjei Mikkelsen (Harvard Stem Cell Institute and Broad Institute). The Mikkelsen lab has developed a massively parallel reporter assay (MPRA) that produces Sort-Seq-like data on transcriptional enhancers in mammalian cell culture. During the last year, I have been working in collaboration with Tiberiu Tesileanu and the Callan lab to analyze the sequence data produced by MPRA.

MPRA was demonstrated on two mammalian enhancers: a cAMP-responsive enhancer (CRE) and the human interferon beta enhancer (IFNβ). By modeling the sequence-dependent activity of these enhancers under both inducing and noninducing conditions, we were able to design new enhancers that have increased inducibility. We expect this work will pave the way for reliable model-based construction of novel human enhancers, expanding the possibilities for synthetic biology.

PUBLICATION

In Press

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Justin Kinney

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 CSHL Fellows Program has been tremendously successful and has served as a paradigm for several analogous programs at other institutions, most recently a Fellows Program sponsored by the National Institutes of Health.

The success of the program is apparent from the list of distinguished alumni. Most notably, Carol Greider—recipient of the 2009 Nobel Prize in Physiology or Medicine for her work on telomerase and telomere function—joined the Fellows Program in 1998. After completing her fellowship, Carol was on the CSHL faculty for 9 years, and she is currently the Daniel Nathans Professor and Director of Molecular Biology and Genetics at Johns Hopkins University School of Medicine.

Previous CSHL Fellows Adrian Krainer (1986) and Marja Timmermans (1998) are now Professors at the Laboratory, and Florin Albeanu (2008) is currently holding an Assistant Professor position in the CSHL neuroscience group. Scott Lowe (1995) is a Howard Hughes Medical Institute (HHMI) Investigator. After nearly 15 years on the CSHL faculty, he recently took on a Professorship at Memorial Sloan-Kettering Cancer Center in New York City. Eric Richards (1989) currently is the Vice President of Research and Senior Scientist at the Boyce Thompson Institute for Plant Research at Cornell University; David Barford (1991) is a Fellow of the Royal Society and Professor of Molecular Biology at the Institute of Cancer Research in London; Ueli Grossniklaus (1994) is Professor at the Institute of Plant Biology, University of Zürich, Switzerland; and Térence Strick (2000) left at the end of his fellowship to become a Group Leader at the Institute Jacques Monod in Paris. 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, Charlottesville. Patrick Paddison, who had joined the Fellows Program in 2004, currently is an Assistant Member at the Fred Hutchinson Cancer Research Center in Seattle, Washington.

Chris Vakoc has been a Fellow at the Laboratory since 2008. He 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 how chromatin regulatory pathways participate in the pathogenesis of cancer.

Mechanistic Studies of the Chromatin Regulatory Machinery in the Hematopoietic System

C.R. Vakoc, J. Lee, J. Molazzo, S. Shareef, J. Shi, M. Taylor, E. Wang

Chromatin, composed of DNA in complex with protein, is the fundamental packaging system for eukaryotic genomes. The basic organizational unit of chromatin is the nucleosome, composed of an octamer of core histones (H2A, H2B, H3, and H4) wrapped by 147 bp of DNA. In addition to protecting the genetic code from genotoxic insult, the structure of chromatin has a profound impact on effectively all DNA-templated processes, including gene transcription, DNA replication, and recombination. Hence, an elaborate regulatory system has evolved to an-

imate chromatin structure so as to facilitate such fundamental cellular activities. Two principal categories of chromatin regulators are the enzymes (e.g., acetyltransferases, deacetylases, methyltransferases, and demethylases) and the reader domain-containing proteins (e.g., bromodomains, chromodomains, and PHD domains). The central long-term goal of research performed in our lab is to explore the mechanistic basis of chromatin regulation and how it relates to the control of gene transcription. We have had a long-standing interest in the relationship between DNA-binding "master-regulatory" transcription factors and the chromatin regulatory machinery, and how this interplay governs cell-fate programs. The biological context in which we study chromatin is in the hematopoietic system, where a hierarchically organized cell lineage program is specified in large part by transcriptional regulators. In addition, studying the hematopoietic systems affords a number of powerful biochemical, as well as genetic, tools for evaluating chromatin and transcription in vivo and ex vivo.

An approach taken by our laboratory for studying transcriptional/chromatin regulators has been to compare their action under normal physiologic settings with their role in pathologic conditions, which can deviate demonstrably from the cellular context under which these components evolved. As an example, normal tissues in vivo generally possess robust gene regulatory networks, such that profound redundancy in gene function can exist under laboratory conditions, which can often obscure studies of molecular mechanisms. In contrast, in aberrant disease states that deviate from normal homeostasis, the nonredundant function of specific regulators can often emerge, thus enabling unique insight into gene function. In addition, studying the action of chromatin regulators in the pathophysiology of disease affords opportunities to translate laboratory observations into biomedical applications, as will be illustrated below. To this end, we have spent the last several years working in close collaboration with Scott Lowe's laboratory at CSHL to investigate the function of chromatin regulators in the setting of hematopoietic cancer—specifically, the disease acute myeloid leukemia (AML). Interestingly, the hematopoietic cell-lineage transcriptional program is often corrupted during leukemogenesis, frequently by direct somatic mutation of transcription factors and chromatin components. The result of this deregulation is an acquired inability of myeloid progenitor cells (a type of white blood cell) to complete terminal differentiation into macrophages and neutrophils, thereby gaining the capability of infinite self-renewal normally seen only in stem cells. We seek to understand how chromatin regulators directly participate in this unique pathophysiology of AML.

Our initial work in this area has focused on two specific chromatin regulators: DOT1L and MLL (mixed-lineage leukemia). DOT1L encodes a histone H3K79 methyltransferase and MLL encodes a histone H3K4 methyltransferase. We have previously published the first epigenomic analysis of DOT1L and H3K79 methylation in mammalian cells, identifying this regulator as a pervasive component of recently initiated transcription elongation complexes. More recently, we also identified and published a novel role for MLL in the epigenetic inheritance of gene expression states during mitosis through a "bookmarking" mechanism. Both MLL and DOT1L have important roles during normal hematopoiesis, but they are also intimately connected to the initiation of AML. As a leukemia proto-oncogene, MLL is frequently mutated via chromosomal translocation to generate oncogenic fusion proteins. Interestingly, MLL–fusion proteins lack their native H3K4 methyltransferase domain and instead directly recruit DOT1L via the carboxy-terminal fusion partner. Hence, MLL fusions lead to the aberrant hypermethylation of H3K79 at many genomic sites, resulting in deregulated transcription programs. MLL fusion leukemia is a widely used experimental model system for studying leukemogenesis as a consequence of epigenetic deregulation.

Using a genetically engineered mouse model of MLL as well as a collection of human leukemia lines, we have sought to identify the essential chromatin regulators necessary to implement the unique hematopoietic phenotype seen in this disease, i.e., the aberrant self-renewal harbored by committed myeloid progenitors. Importantly, the MLL–leukemia mouse model recapitulates many of the aggressive features of the human disease, including resistance to cytotoxic chemotherapy. Hence, identifying chromatin regulators as dependencies in this model can be used as preclinical proof-of-concept for discovering and validating novel drug targets for the human disease.

Identification of the Bromodomain Protein BRD4 as a Leukemia Maintenance Gene, Upstream Regulator of MYC Expression, and Drug Target in AML

In an initial effort to systematically identify chromatin regulatory, or "epigenetic," dependencies in MLL, we conducted a short hairpin RNA (shRNA) screen targeting ~250 of the most well-documented chromatin machineries, including most known writers, readers, and

erasers of chromatin modifications. All chromatin regulators were systematically suppressed and scored for their impact on growth of MLL cultures ex vivo. Among the genes identified in the screen, BRD4 was among the most striking, based on the overall strength of the growth-inhibitory phenotype observed following shRNA knockdown. Importantly, BRD4 knockdown suppressed growth of a multitude of different mouse and human AML lines, including, but not limited to, those harboring MLL–fusion oncogenes. BRD4 inhibition, when delivered conditionally to established leukemias in vivo, could also attenuate disease progression. Thus, our RNA interference (RNAi) screen identified BRD4 as a unique dependency in AML, which had no prior links to hematopoietic cancer pathogenesis.

Convergent studies from the chemistry laboratory of Dr. James Bradner at the Dana-Farber Cancer Institute identified a first-in-class small-molecule inhibitor of BRD4, called JQ1, with demonstrated preclinical activity in suppressing growth of BRD4-NUT squamous cell carcinoma. Through collaborative studies performed with the Bradner laboratory, we observed that JQ1 also dramatically attenuated progression of the MLL–fusion leukemia mouse model. Remarkably, JQ1 treatment leads to a phenotype overtly similar to BRD4 knockdown, as revealed by flow cytometry analysis of cell-surface markers, expression microarrays, and cell morphology, as well as a broad antileukemia activity in a collection of different AML lines. Together, these findings revealed that small-molecule inhibition of BRD4 is an effective pharmacological strategy in suppressing leukemia progression.

Subsequent mechanistic studies revealed that one of the most prominent consequences of BRD4 inhibition, whether with shRNAs or JQ1, was the suppression of MYC RNA and protein levels. *MYC* is a well-known oncogene with an established role in initiation and progression of a broad variety of cancer types. Remarkably, exposing cells to JQ1 led to a nearly 50-fold suppression of MYC expression (see Fig. 1). By ectopically expressing MYC from an artificial transgene, we demonstrated that MYC suppression accounts significantly for the antileukemia effects of JQ1 or BRD4 shRNA. Thus, BRD4 provides an opportunity for small-molecule inhibition of MYC at the level of expression by inhibiting its bromodomain function with JQ1.

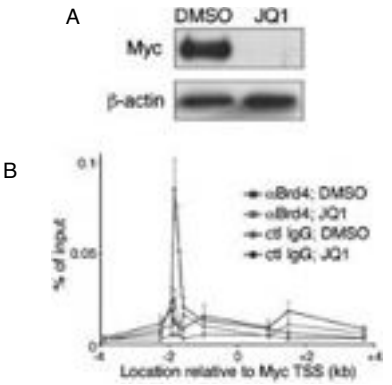

Figure 1. (*A*) Western blotting of whole-cell lysates prepared from MLL-AF9/Nras^{G12D} leukemia cells treated for 48 h with DMSO or 250 nM JQ1.(*B*) ChIP–qPCR performed in MLL cells with indicated antibodies and PCR primer locations. (TSS) Transcription start site.

Ongoing studies in the laboratory are now focused on identifying other components of the BRD4 pathway that might be involved in leukemia pathogenesis, as well as continuing studies on epigenetic inheritance through mitosis. Clinical trials in leukemia patients with drug-like derivatives of JQ1 will be initiated within the next year by Tensha Therapeutics.

PUBLICATIONS

Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastritis E, Gilpatrick T, Paranal RM, Qi J, et al. 2011. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. *Cell* **146:** 904–917.

Zuber J, Rappaport AR, Luo W, Wang E, Chen C, Vaseva AV, Shi J, Weissmueller S, Taylor MJ, Weissenboeck M, et al. 2011. An integrated approach to dissecting oncogene addiction implicates a Myb coordinated self-renewal program as essential for leukemia maintenance. *Genes Dev* **25:** 1628–1640.

Zuber J, Shi J, Wang E, Rappaport AR, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, et al. 2011. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukemia. *Nature* **478:** 524–528.

AUTHOR INDEX

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WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

The Watson School of Biological Sciences (WSBS) continues to be one of the most successful Ph.D. programs in the United States. Our time to graduation is ~3 years faster than the national average, the publication record of our students is outstanding, and the success of our alumni is remarkable.

Team Watson: (*Left to right*) Keisha John, Dawn Meehan, Kim Geer, Leemor Joshua-Tor, and Alyson Kass-Eisler

Dr. Keisha John has been promoted to Associate Director for Recruitment and Undergraduate Research at the WSBS. Keisha is an alumna of the WSBS graduate and URP (Undergradate Research Program) programs. An invaluable member of "Team Watson," working closely with Dawn, Alyson, and Kim, she has been coordinating all visiting students at the Laboratory and is heavily involved in recruitment for our graduate and undergraduate programs. One of Keisha's new responsibilities is the administration of the URP, and together with URP Director Professor Zachary Lippman, she has created a fantastic environment of science and fun over the summer.

Faculty Changes

One new faculty member joined the Watson School in 2011: Christopher Vakoc. Chris, an Assistant Professor, first came to Cold Spring Harbor Laboratory (CSHL) in 2008 as a Cold Spring Harbor Fellow and was promoted to the WSBS faculty this year. His work focuses on how chromatin regulatory pathways participate in the pathogenesis of cancer. As a fellow, Chris has already participated in School activities as a course lecturer, examiner on qualifying exams, and thesis committee member. We look forward to his growing participation as a core member of the faculty.

The Eighth WSBS Graduation

On May 1, we celebrated the Watson School's eighth graduation ceremony. Two students, Amy Rappaport and Claudio Scuoppo, both from the Entering Class of 2006, were awarded the Ph.D. degree. Jiahao Huang from the Entering Class of 2008 was awarded a Masters degree, but did not attend the ceremony. Honorary degrees were bestowed upon Dr. James Simons and Dr. James Lupski. Dr. Simons is the former Chairman of the Mathematics Department at Stony Brook University and current Board Chair of Renaissance Technologies LLC. He is credited with the discovery and application of certain geometric measurements used in theoretical physics, particularly string theory, known as the Chern–Simons Invariants. Dr. Simons and his wife also manage the Simons Foundation, whose philanthropic activities include the establishment of an institute for research in mathematics and theoretical physics and a major research initiative on the causes of autism. He was honored as a scholar and tireless advocate of education, mathematics, and scientific research. Dr. James Lupski was an Undergraduate Research Participant (URP) at CSHL in the laboratory of Dr. Ahmad Bukhari during the summers of 1978 and 1979 and is currently a Professor at the Baylor College of Medicine. He is an accomplished scientist who has earned national distinction for his groundbreaking work in "genomic disorders," a term he coined. Nearly two decades ago, he identified the chromosome 17p12 duplication in the nerve disorder, Charcot–Marie–Tooth syndrome type 1A (CMT1A), a disease he himself is afflicted with. He was honored as a medical practitioner

2011 graduates: (*Left to right*) Honorary degree recipient James Simons, WSBS Dean Leemor Joshua-Tor, Amy Rappaport, Claudio Scuoppo, CSHL Board of Trustees Chair Jamie Nicholls, CSHL President Bruce Stillman, honorary degree recipient James Lupski

and pioneering researcher, who has earned distinction as one of the premier researchers of genomic disorders of our time. Dr. Lupski also delivered a heartfelt and inspiring commencement address.

As with each graduation, we extended a special welcome to the family members and friends of our students who attend the ceremony. Among these special guests were Claudio's family members who traveled from Italy to take part in this special event.

Teaching Award

Michael Schatz

At this year's graduation, Michael Schatz was presented with the sixth annual Winship Herr Faculty Teaching Award named in honor of the School's founding dean. Michael was an instructor in our brand-new Specialized Disciplines course in Quantitative Biology. He was chosen by the students for this award, based on his enthusiasm, excellence, and creativity in teaching. The winner of this award is nominated and voted on by the students. Below is some of what the students had to say about Michael:

> *Michael Schatz showed the utmost patience with students trying to learn extremely challenging material. He used cleverly thought-out analogies to explain complicated concepts rather easily and he was an engaging lecturer who was able to extend his passion and enthusiasm for the subject to the students.*

Admissions 2011

The School received 256 applications for the 2011/2012 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 2011 entering class comprised Gregory Hannon (chair), Mickey Atwal, Leemor Joshua-Tor, Adam Kepecs, Adrian R. Krainer, Zachary Lippman, W. Richard McCombie, Stephen Shea, Nicholas Tonks, and Linda Van Aelst—a truly remarkable team!

2011 WSBS DOCTORAL DEGREE RECIPIENTS

Student	Thesis advisor	Academic mentor	Current position
Patrick Finigan	Robert Martienssen	Senthil K. Muthuswamy	Postdoctoral fellow with Dr. Robert Martienssen, CSHL
Amy Rappaport	Scott Lowe	Marja Timmermans	Postdoctoral fellow with Dr. Leisa Johnson, Genentech, San Francisco, California
Frederick Rollins	Gregory Hannon	Jan. A. Witkowski	Postgraduate student with Dr. Gregory Hannon, CSHL
Claudio Scuoppo	Scott Lowe	Gregory Hannon	Postdoctoral fellow with Dr. Riccardo Dalla-Favera, Columbia University, New York

2011 THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2005

Patrick Finigan, July 15, 2011

The origin of novel phenotypic variation in Arabidopsis *allopolyploids.*

Thesis Examining Committee

Chair:	**Zachary Lippman**
Research mentor:	**Robert Martienssen**
Academic mentor:	**Senthil K. Muthuswamy**
Committee member:	**David Jackson**
Committee member:	**Doreen Ware**
Committee member:	**Eric Richards,** *Thompson Institute for Plant Research*
External examiner:	**Craig Pikaard,** *Indiana University*

Frederick Rollins, July 6, 2011

The genetics and epigenetics of erlotinib resistance in non-small-cell lung cancer.

Thesis Examining Committee

Chair:	**Raffaella Sordella**
Research mentor:	**Gregory Hannon**
Academic mentor:	**Jan A. Witkowski**
Committee member:	**Scott Lowe**
Committee member:	**Scott Powers**
External examiner:	**Charles L. Sawyers,** *Memorial Sloan-Kettering Cancer Center*

ENTERING CLASS OF 2006

Amy Rappaport, March 17, 2011

Dissecting tumor suppressor and tumor maintenance genes in poor prognosis AML.

Thesis Examining Committee

Chair:	**Gregory Hannon**
Research mentor:	**Scott Lowe**
Academic mentor:	**Marja Timmermans**
Committee member:	**Bruce Stillman**
Committee member:	**Lloyd Trotman**
Committee member:	**Jan A. Witkowski**
External examiner:	**Kevin M. Shannon,** *University of California, San Francisco*

Claudio Scuoppo, March 21, 2011

Architectural models of tumor suppression in lymphoma.

Thesis Examining Committee

Chair:	**Senthil K. Muthuswamy**
Research mentor:	**Scott Lowe**
Academic mentor:	**Gregory Hannon**
Committee member:	**Scott Powers**
Committee member:	**Lloyd Trotman**
Committee member:	**Raffaella Sordella**
External examiner:	**Carol Prives,** *Columbia University*

ENTERING CLASS OF 2007

Wang Zhenxun, December 13, 2011

The mechanism and manipulation of PK-M alternative splicing.

Thesis Examining Committee

Chair:	**Leemor Joshua-Tor**
Research mentor:	**Adrian R. Krainer**
Academic mentor:	**Terri Grodzicker**
Committee member:	**Gregory Hannon**
Committee member:	**Darryl Papin**
External examiner:	**Brenton R. Gravely,** *University of Connecticut Health Center*

DOCTORAL THESIS RESEARCH

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2006			
Eyal Gruntman *Elisabeth Sloan Livingston Fellow*	Josh Dubnau	Glenn Turner	Olfactory perception in *Drosophila*.
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 hot spots: Characterizing fine-scale variation in the frequency of meiotic recombination across the mammalian genome.
Joseph Calarco *NSERC Scholar* *David H. Koch Fellow*	David Jackson	Robert Martienssen	Transposable element (TE) regulation in the flowering plant *A. thaliana*.
Saya Ebbesen *NIH Predoctoral Trainee (ARRA)* *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* *NIH Predoctoral Trainee* *William Randolph Hearst Student*	Adrian R. Krainer	Gregory Hannon	Dissecting the piRNA pathway in the *Drosophila* ovarian somatic sheet.
Kyle Honegger *Crick-Clay Fellow* *NIH Predoctoral Trainee*	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	CLIP method as a way of direct readout of microRNA target sites.
Hassana Oyibo *Farish-Gerry Fellow* *William Randolph Hearst Student*	Hiro Furukawa	Anthony Zador	Reconstruction of connectivity of neurons by reading of oligonucleotide labels (ROC-N-ROL).
Michael Pautler *NSERC Scholar* *William R. Miller Fellow*	Robert Lucito	David Jackson	The RAMOSA pathway and inflorescence branching in maize.
Maria Pineda *William Randolph Hearst Student* *CSHL Women's Partnership for Science Student*	Adrian R. Krainer	Raffaella Sordella	Mechanism of "addiction" to receptor tyrosine kinases in non-small-cell lung carcinoma.
Yevgeniy Plavskin *Alfred Hershey Fellow* *NIH Predoctoral Trainee*	Jan A. Witkowski	Marja Timmermans	The evolution of the miR390-dependent tasiRNA pathway and its function in bryophyte development.
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* Thesis defense: December 2011	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*	Alexander Gann	Thomas Gingeras	Transposons and evolution of transcriptional regulation in the *Drosophila* clade.
Mitchell Bekritsky *Starr Centennial Scholar*	W. Richard McCombie	Michael Wigler	High-throughput microsatellite genotyping.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Dario Bressan *Robert and Teresa Lindsay Fellow*	Z. Josh Huang	Gregory Hannon	A genomic approach toward the eludication of connectivity patterns at cellular resolution in complex neural networks.
Melanie Eckersley-Maslin *Genentech Foundation Fellow*	Gregory Hannon	David L. Spector	Making the choice: Mechanistic insights into random autosomal monoallelic expression in mammalian cells.
Sang Geol Koh *George A. and Marjorie H. Anderson Fellow*	Glenn Turner	Anthony Zador	Attention-dependent information routing in the mouse auditory cortex.
Katie Liberatore *Starr Centennial Scholar* *National Science Foundation Predoctoral Fellow*	Adrian R. Krainer	Zachary Lippman	Inflorescence development and heterosis.
Ozlem Mert *George A. and Marjorie H. Anderson Fellow*	John R. Inglis	Scott Lowe	Characterization of the role of E2F7 in oncogene-induced senescence and tumorigenesis.
Elizabeth Nakasone *William Randolph Hearst Fellow* *Leslie C. Quick, Jr. Fellow*	Alea A. Mills	Mikala Egeblad	Understanding the effects of cytotoxic chemotherapeutics on the innate immune response.
Zinaida Perova *Charles A. Dana Fellow*	Linda Van Aelst	Bo Li	The role of medial prefrontal cortex in behavioral depression.
Felix Schlesinger *Crick-Clay Fellow*	Gregory Hannon	Thomas Gingeras	Classification of novel transcription start sites.
Nilgun Tasdemir *Robert and Teresa Lindsay Fellow II*	Josh Dubnau	Scott Lowe	Investigating the molecular overlaps between epigenetic reprogramming and transformation.
Elvin Wagenblast *Starr Centennial Scholar* *Boehringer Ingelheim Fellow*	Jan A. Witkowski	Gregory Hannon	Role of stem/progenitor cells in mammary gland and breast tumors.
Susann Weissmueller *Annette Kade Fellow*	Raffaella Sordella	Scott Lowe	In vivo identification and characterization of tumor suppressor genes in hepatocellular carcinoma.
ENTERING CLASS OF 2009			
Stephane Castel *Cashin Fellow* *NSERC Scholar*	Lloyd Trotman	Robert Martienssen	RNAi-mediated heterochromatin in *S. pombe*.
Kristen Delevich *NIH Predoctoral Trainee*	Stephen Shea	Bo Li	Elucidating the role of *Disrupted-in-Schizophrenia-1* in development of prefrontal cortical circuits.
Silvia Fenoglio *Elisabeth Sloan Livingston Fellow*	Linda Van Aelst	Gregory Hannon	RNAi screening to identify putative therapeutic targets for the treatment of pancreatic cancer.
Wee Siong Goh *A*STAR Fellow* *Delbrück Fellow*	Hiro Furukawa	Gregory Hannon	Determining piRNA primary biogenesis, and MIWI and late piRNA function in mice using *C. elegans* as a model system for genetic screening.
Ian Peikon *Dr. John and Consuelo Phelan Student*	Mickey Atwal	Anthony Zador	Reverse engineering the brain.
Kaja Wasik *George A. and Marjorie H. Anderson Fellow*	Jan A. Witkowski	Gregory Hannon	A screen for novel components of the piRNA pathway in *D. melanogaster*.
Cinthya Zepeda Mendoza *Gonzalo Rio Arronte Fellow*	Thomas Gingeras	David L. Spector	Analysis of higher-order chromatin organization at the mouse syntenic region of human 1p36 upon genomic copy-number changes.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2010			
Arkarup Bandyopadhyay *Goldberg Lindsay Fellow* Proposal defense: January 2012	Zachary Lippman	Florin Albeanu	Identity and intensity encoding of odors in rodents.
Colleen Carlston *John and Amy Phelan Student* Proposal defense: January 2012	Alexander Gann	Christopher Hammell	Identification and characterization of noise-suppressor genes that act via microRNAs in *C. elegans* larval development.
Matthew Koh *George A. and Marjorie Anderson Fellow* Proposal defense: January 2012	Bo Li	Florin Albeanu	Roles of olfactory bulb inhibitory microcircuits in shaping the temporal response properties of mitral cells.
Lisa Krug *NIH Predoctoral Trainee* Proposal defense: January 2012	Stephen Shea	Josh Dubnau	Mechanisms of transposon regulation in the central nervous system.
John Sheppard *Bristol Myers Squibb/NIH Predoctoral Trainee* Proposal defense: January 2012	Josh Dubnau	Anne Churchland	Neural mechanisms of multisensory decision-making.
Jack Walleshauser *Barbara McClintock/NIH Predoctoral Trainee* Proposal defense: January 2012	Christopher Hammell	Leemor Joshua-Tor	Structural basis for TUT4 uridylation of pre-let-7/lin28 complex.

Entering Class of 2011

On August 29, 2011, the Watson School opened its doors for the 13th time to welcome yet another new class. This year, 10 students joined the School: Robert Aboukhalil, Brittany Cazakoff, Maria Joaquina Delas Vives, Anja Hohmann, Justus Kebschull, Fred Marbach, Onyekachi Odoemene, Sophie Thomain, Charles Underwood, and Taekyung (Daniel) Yun. Fred and Sophie were visiting Master's students in Florin Albeanu and Robert Martienssen's laboratories, respectively, in 2010. Robert was a participant in the School's Undergraduate Research Program in 2010 under the mentorship of Mickey Atwal.

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. Because WSBS students select a research mentor only in June right before their qualifying exam, it is the unique responsibility of the academic mentor to monitor students—and offer advice—during the intensive coursework of the first term, during their rotations, and to help them identify a suitable research mentor. They then follow them throughout their doctoral experience, often serving as important advocates for the students. Entering students select by mutual agreement a member of the research or nonresearch faculty to serve as an academic mentor. This program continues to receive much support from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in our success. The following are this year's new Academic Mentors for the Entering Class of 2011:

STUDENT	MENTOR	STUDENT	MENTOR
Robert Aboukhalil	Josh Dubnau	Fred Marbach	Josh Dubnau
Brittany Cazakoff	Christopher Hammell	Onyekachi Odoemene	Stephen Shea
Maria Joaquina Delas Vives	Nicholas Tonks	Sophie Thomain	Zachary Lippman
Anja Hohmann	John Inglis	Charles Underwood	Alex Gann
Justus Kebschull	Marja Timmermans	Taekyung (Daniel) Yun	Raffaella Sordella

ENTERING CLASS OF 2011

Robert Aboukhalil, McGill University; Cold Spring Harbor Laboratory Summer Undergraduate Research Program (2010); Technophilic Magazine: Publication of the Year (2010); CS Games 2009 and 2010: Member of McGill team that ranked second in North American programming competition (2009, 2010); McGill Faculty of Science Student Research Ambassador (2009); CAE Scholarship in Engineering Excellence (2009); Louis C. Ho Scholarship in Engineering (2008); McGill University Dean's Honour List (2008); Canada-wide Science Fair (2007)
Academic Mentor: Josh Dubnau

Brittany Cazakoff, University of Saskatchewan; Marine Biological Lab, Woods Hole, MA: Neural Systems and Behavior Course (2010); University of Saskatchewan Graduate Scholarship (2010); Orange Benevolent Society Scholarship (2010); Natural Sciences and Engineering Research Council of Canada (NSERC); Alexander Graham Bell Canada Graduate Scholarship (2009); Governor General's Silver Medal (2009)
Academic Mentor: Christopher Hammell

Maria Joaquina Delas Vives, Universidad Politecnica de Valencia; Erasmus Placement Scholarship: German; Cancer Research Center (DKFZ): Heidelberg, Germany (2010); Spanish National Research Council Grant: Centro de Biología Molecular "Severo Ochoa" (CBMSO). Madrid, Spain (2009); Spanish Ministry of Science Grant Recipient (2009); Valencia iGEM Team: Gold Medal (2008, 2009); Experimental Science in Pablo de Olavide University: Oral Presentation 2nd Prize (2008)
Academic Mentor: Nicholas Tonks

Anja Hohmann, University of Cambridge; B.A. and M.Sci. Awarded: University of Cambridge (2011); Cambridge iGEM Team: Finalist, "Best Wiki" and Gold Medal Awarded (2010); Hal Dixon Trust Research Grant (2010); The George Mallory Prize: University of Cambridge (2009); David Hendersen Memorial Trust Fund Travel Grant (2008); Cambridge European Trust Bursary (2007–2011)
Academic Mentor: John R. Inglis

Justus Kebschull, University of Cambridge; B.A. and M.Sci. Awarded: University of Cambridge (2011); German Society for Periodontology Travel Award (2010); Trinity College Summer Research Studentship (2010); Senior Scholarship, Trinity College (2009, 2010); University of Cambridge/Massachusetts Institute of Technology Exchange (2009–2010); Junior Scholarship, Trinity College (2008)
Academic Mentor: Marja Timmermans

Fred Marbach, Swiss Federal Institute of Technology (EPFL); M.S. Awarded: Swiss Federal Institute of Technology (EPFL) (2010)
Academic Mentor: Josh Dubnau

Onyekachi Odoemene, The George Washington University; M.S. Awarded: Purdue University (2011); Honorable Mention, NSF Graduate Research Fellowship (2010); Carl Storm Travel Fellowship (2010); Purdue University Doctoral Fellowship (2009); Deans List: The George Washington University (2005–2009); Coulter Foundation Summer Research Grant (Summer 2007); George Gamow Research Fellowship (2006–2007); Howard Hughes Summer Research Scholar (Summer 2006)
Academic Mentor: Stephen Shea

Sophie Thomain, Ecole Normale Superieure; M.S. Awarded: Ecole Normale Superieure (2011); Amgen Foundation Scholarship: University of Cambridge (Summer 2009)
Academic Mentor: Zachary Lippman

Charles Underwood, University of Oxford; Academic Merit Scholarship: St Anne's College, University of Oxford (2008–2011); Summer Studentships: Cancer Research UK (2010), Amgen Foundation (2009); Ludwig-Maximilians-University of Munich (2009); BP Ultimate Field Trip Challenge Finalist (2010); Travel Grant: St. Anne's College, University of Oxford (2009)
Academic Mentor: Alexander A.F. Gann

Taekyung Yun, The Johns Hopkins University; M.A. Awarded: Columbia University (2010)
Academic Mentor: Raffaella Sordella

2011 entering class: (*Front row, left to right*) Anja Hohmann, Brittany Cazakoff, Sophie Thomain, Joaquina Delas Vives. (*Back row, left to right*) Fred Marbach, Robert Aboukhalil, Daniel Yun, Charles Underwood, Justus Kebschull, Kachi Odoemene

The Fall Term Curriculum

Our faculty continues to do an outstanding job of developing and delivering the curriculum. We are extremely grateful for their considerable time and effort in maintaining the high-quality coursework that we strive to provide. The Curriculum Development and Integration Committee (CDIC)—Adrian Krainer (chair), David Jackson, Leemor Joshua-Tor, Nicholas Tonks, and Glenn Turner—continues to carefully monitor and develop the curriculum. In addition to the outstanding course instructors and guest lecturers from within the Laboratory, our courses also continue to attract an impressive array of guest lecturers from other institutions.

Recruiting Efforts

Recruitment for the graduate program's 2011 class and our URP 2011 was once again managed by Ms. Dawn Pologruto, the School's Director for Admissions and Student Affairs. As in years past, Dawn, our faculty, and our students traveled the length and breadth of the country representing CSHL and WSBS. They were joined by Keisha John who did most of the traveling this year, making special recruitment visits aimed at enhancing diversity. Keisha also stepped in to oversee the last two interview weekends when Dawn went out on maternity leave in February. The table beginning on the next 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

WSBS students account for approximately half of the total graduate students here at CSHL. The other half of the students are visiting graduate students from other universities who have decided to conduct some or all of their thesis research in CSHL faculty members' laboratories. A large percentage of students are from Stony Brook University (SBU), via a program established between CSHL and SBU more than 30 years ago. Over the years, we have also established relationships with institutions around the world, thereby enabling their students to conduct research here at CSHL. Currently, we have visiting students from institutions in Germany, France, Mexico, and India to name a few. Keisha John serves as the visiting student liaison in the WSBS. In this capacity, she is the onsite contact person for the students, and she maintains relationships with administrations from their home institutions. These students are fully integrated into the CSHL community and receive all the necessary assistance as they navigate the complexities of performing doctoral research away from their home institutions. The following students, listed in the box below, joined us this year.

Graduate Student Symposium

Each year, the students participate in three Graduate Student Symposia held at the Laboratory's Genome Research Center in Woodbury: one each in January, May, and October. Each Symposium consisted of five sessions where all of the senior students gave 15-minute talks. Coffee breaks, lunch, and a wine and cheese reception at the end of the day rounded out the program and provided opportunities for more informal interactions. The Graduate Student Symposium is open to the entire Laboratory community, and as in previous years, all in attendance found the Symposia to be very worthwhile. We are grateful to the two student chairs of the Symposium, Dong-Woo Hwang (SBU) and Mitchell Bekritsky (WSBS) and to Keisha John for providing School oversight.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL research mentor	Affiliation and program
Michelle Falerio	Adam Kepecs	Universitat Pompeu Fabra, Biomedicine, Spain
Zhen Gong	Partha Mitra	Stony Brook, Biomedical Engineering
Priyanka Gupta	Florin Albeanu	National Centre for Biological Sciences, Neuroscience, India
Hyun-Yong Jeon	Adrian Krainer	Stony Brook, Molecular and Cellular Biology
Sean M. Kelly	Z. Josh Huang	Stony Brook, Medical Scientists Training Program
Ha Yan Lee	Michael Schatz	Stony Brook, Computer Science
Ioana A. Rus	Nick Tonks	Stony Brook, Genetics
Siwei Zhang	Nick Tonks	Stony Brook, Microbiology

2011 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS attendees/titles
American Association of Cancer Research Annual Meeting	Orlando, Florida	April 2–6	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
New York Institute of Technology Career and Internship Fair	New York Institute of Technology	April 7	Keisha John, Associate Director of Recruitment and Undergraduate Research
Experimental Biology Annual Meeting	Washington, D.C.	April 9–13	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
American Society for Microbiology Annual Meeting	New Orleans, Louisiana	May 20–24	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
International RNA Society Meeting	Kyoto, Japan	June 14–18	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
International Society for Stem Cell Research Meeting	Toronto, Canada	June 15–18	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
New York City College of Technology NSF STEP Program Visit	Cold Spring Harbor Laboratory	July 14	Kristen Delevich, Graduate Student Dawn Pologruto
University of Medicine and Dentistry, New Jersey: Summer Internship Program Visit	Cold Spring Harbor Laboratory	July 15	Michael Pautler, Graduate Student Dawn Pologruto
LaGuardia Community College Visit	Cold Spring Harbor Laboratory	July 25	Hassana Oyibo, Graduate Student
American Society of Plant Biologists Annual Meeting	Minneapolis, Minnesota	August 8–11	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
Brookhaven National Laboratory: Graduate School Fair and Undergraduate Research Program Symposium	Brookhaven National Laboratory	August 10	Keisha John
Williams College: Information Session	Williams College	September 16	Keisha John
University of Maryland, Baltimore County: Meyerhoff Scholarship Program and MARC Program Visit	University of Maryland, Baltimore County	September 19	Keisha John
Cornell University: Information Session	Cornell University	September 20	Ian Peikon, Graduate Student Kaja Wasik, Graduate Student
The Johns Hopkins University: Information Session	The Johns Hopkins University	September 20	Keisha John
Massachusetts Institute of Technology Career Fair	Massachusetts Institute of Technology	September 21	Dawn Pologruto
Cornell University: Graduate and Professional School Day	Cornell University	September 21	Ian Peikon Kaja Wasik

2011 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE (*continued*)

Event	Location	Date	WSBS attendees/titles
Morgan State University: MBRS-RISE Program: Graduate Career Workshop and Information Session	Morgan State University	September 21	Keisha John
Hunter College Minority Access for Research Careers (MARC) Program: Information Session	Hunter College	September 21	Kristen Delevich Stephen Shea, Assistant Professor
University of Maryland, Eastern Shore MARC Program: Information Session	University of Maryland, Eastern Shore	September 22	Keisha John
Howard University: Information Session	Howard University	September 22	Keisha John
Massachusetts Institute of Technology: Information Session	Massachusetts Institute of Technology	September 23	Dawn Pologruto
University of Maryland, College Park: Information Session	University of Maryland, College Park	September 23	Keisha John
Big 10+ Graduate School Fair	Purdue University	September 26	Colleen Carlston, Graduate Student
Duke University: Information Session	Duke University	September 27	Stephen Shea
University of California, Santa Cruz MARC Program: Information Session	University of California, Santa Cruz	September 28	Keisha John
The Johns Hopkins University Career Fair	The Johns Hopkins University	September 28	Hassana Oyibo, Graduate Student
University of California, San Diego MARC Program: Information Session	University of California, San Diego	October 3	Keisha John
University of California, Berkeley: Graduate School Fair	University of California, Berkeley	October 5	Keisha John
Stanford University: Information Session	Stanford University	October 5	Keisha John
Xavier University: Research Talk and MARC Program Information Session	Xavier University	October 6	Alea Mills, Professor
Princeton University: Information Session	Princeton University	October 6	Keisha John
Northwestern University: Information Session	Northwestern University	October 7	Petr Znamenskiy, Graduate Student
Princeton University Graduate and Professional School Fair	Princeton University	October 7	Keisha John
University of New Mexico: Information Session	University of New Mexico	October 7	Katie Liberatore, Graduate Student
Brooklyn College of CUNY MARC and RISE Program Information Session	Brooklyn College of CUNY	October 11	Keisha John
International Congress of Human Genetics Meeting	Montreal, Canada	October 11–15	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
St. John's University: Information Session and Campus Visit	Cold Spring Harbor Laboratory	October 12	Zina Perova, Graduate Student Dawn Pologruto
Morehouse College: Information Session	Morehouse College	October 14	Keisha John
Wellesley College: Information Session	Wellesley College	October 14	Anne Churchland, Assistant Professor
Conference of Research Experiences for Undergraduate Student Scholarship	Arlington, Virginia	October 16–17	Keisha John
Stanford University: Graduate School Fair	Stanford University	October 17	David Simpson, WSBS Alumni
SUNY, Old Westbury: Information Session	SUNY, Old Westbury	October 18	Keisha John
California Institute of Technology Career Fair	California Institute of Technology	October 19	Katie Liberatore
Queens College, City University of New York: MARC-USTAR Program Information Session	Queens College, City University of New York	October 19	Keisha John
Emory University Graduate School Fair	Emory University	October 20	Wee Siong Goh, Graduate Student
Yale University: Information Session	Yale University	October 20	Keisha John
Harvard University: Information Session	Harvard University	October 21	Keisha John
Barry University MARC Program: Information Session	Barry University	October 21	Maria Pineda, Graduate Student
State University of New York, Farmingdale: Information Session and Campus Visit	Cold Spring Harbor Laboratory	October 21	Dawn Pologruto Hassana Oyibo

2011 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE (*continued*)

Event	Location	Date	WSBS attendees/titles
University of Pennsylvania: Information Session	University of Pennsylvania	October 24	Dawn Pologruto
Mount St. Mary's College MARC Program: Information Session	Mount St. Mary's College	October 24	Keisha John
University of California, Irvine MARC and RISE Programs: Information Session	University of California, Irvine	October 25	Keisha John
University of California, Davis BUSP Program: Information Session	University of California, Davis	October 25	Keisha John
University of California, Los Angeles Graduate School Fair	University of California, Los Angeles	October 26	Keisha John
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)	San Jose, California	October 27–29	Melanie Eckersley-Maslin, Graduate Student Keisha John Adrian Krainer, Professor
California Forum for Diversity in Graduate Education: Graduate School Fair	University of San Francisco	October 29	Keisha John
Brown University: Information Session	Brown University	October 31	Keisha John
University of Puerto Rico, Rio Piedras MARC Program: Information Session	University of Puerto Rico, Rio Piedras	November 2	Paloma Guzzardo, Graduate Student
Hofstra University: Information Session	Hofstra University	November 2	Keisha John
Rutgers University: Graduate and Professional School Day	Rutgers University	November 2	Saya Ebbesen, Graduate Student
North Carolina Central University RISE Program: Information Session	North Carolina Central University	November 3	Keisha John
Truman State University: Information Session	Truman State University	November 4	Keisha John
"What Can You Be With a Ph.D.?" Career Convention	New York University	November 5–6	Information sent for distribution
Washington University, St. Louis: Information Session	Washington University, St. Louis	November 8	Keisha John
Annual Biomedical Research Conference for Minority Students (ABRCMS)	St. Louis, Missouri	November 9–12	Arkarup Bandyopadhyay, Graduate Student Colleen Carlston Christopher Hammell, Assistant Professor Brittany Haugen, URP 2011 Keisha John
Sigma Xi Annual Conference and Research Symposium	Raleigh, North Carolina	November 10–12	Maria Pineda
Society for Neuroscience Annual Meeting	Washington, D.C.	November 12–16	CSHL Meetings and Courses and Press personnel attended with WSBS information for distribution
Hunter College Graduate and Professional School Fair	Hunter College	November 15	Joseph Calarco, Graduate Student Keisha John
Columbia University: Information Session	Columbia University	November 15	Keisha John
City College of New York, York College Career and Graduate School Fair	CUNY, York College	November 16	Keisha John
Queensborough Community College, City College of New York: Internship Fair	Queensborough Community College	November 16	Information sent for distribution
George Washington University: Information Session	George Washington University	November 16	Keisha John
Hampton University MARC Program: Information Session	Hampton University	November 17	Keisha John
American Society for Cell Biology Annual Meeting	Denver, Colorado	December 3–7	CSHL Meetings and Courses and Press personnel attended with WSBS information 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 2011:

Postdoctoral Fellows

Benoit Boivin	Ingrid Ibarra	Yuntao Mao	Marlies Rossmann
Maia Chanrion	Douglas Johnston	Sundarmurthy Pandurangan	Man-Hung Eric Tang
Sung Wook Chi	Lolahon Kadiri	Smitha Reddy	Milos Tanurdzic
Yu-Chen Chien	Kannan Krishnamurthy	Francesc Roca	Xianfeng Xu
Alexander Goldschmidt	Jayant Kulkarni	Adam Rosebrock	Miguel Zaratiegui Biurrun

Graduate Students

Sihem Cheloufi	Yu Fu	Mark Orcholski	Yang Yang
Carrie Clandaniel	Lin Guang	Prem Premsrirut	Jianping Zhang
Noelle Cutter	Aftabul Haque	Amy Rappaport	Rui Zhao
Christof Fellmann	Wen Huang	Claudio Scuoppo	
Xin Feng	Matthew Lazarus	Rahul Sinha	
Oliver Fregoso	Zhijie Liu	Elena Tonin	

Executive Committee

The School's Executive Committee, in its monthly meetings, provides year-round direction for the School and its students through its invaluable policy recommendations. I thank all of the faculty members who served on the Executive Committee through 2011: Mickey Atwal, David Stewart, Adrian Krainer, Alea Mills, David Spector, and Linda Van Aelst. Alea Mills completed her second term in December 2011. During her tenure, she contributed tremendously to the committee. There was also turnover among the student representatives as SBU representative Matthew Camiolo was replaced by Deblina Chatterjee, and Mitchell Bekritsky replaced WSBS representative Katie Liberatore. We are thankful for Matthew and Katie who were very insightful and served as wonderful mouthpieces for their colleagues.

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. Nobel laureate Dr. Sydney Brenner, from the Janelia Farm Research Campus of the Howard Hughes Medical Institute and the Salk Institute, was this year's Gavin Borden Fellow. His lecture "Reading the human genome" was well accepted and provoked many questions from the audience. In addition, the students joined Dr. Brenner during a roundtable lunch and discussion where he shared stories from "*A Life in Science*."

The Watson School Continues to Benefit from Generous Benefactors

We are extremely grateful for the generous donors, whose one-time gifts or continued support made our 2011 programs possible, including The Banbury Fund, Bristol-Myers Squibb Corporation, Mr. and Mrs. Richard M. Cashin, Mr. and Mrs. Landon Clay, Lester Crown, The Dana Foundation,

The Samuel Freeman Charitable Trust, The William Stamps Farish Fund, The Genentech Foundation, Mr. and Mrs. Alan Goldberg, Florence Gould Foundation, William Randolph Hearst Foundation, Annette Kade Charitable Trust, Mr. David H. Koch, Mr. and Mrs. Robert D. Lindsay and Family, Mr. and Mrs. David Luke III, Mr. and Mrs. William R. Miller, OSI Pharmaceuticals Foundation, Estate of Edward L. Palmer, Mr. and Mrs. John C. Phelan, The Quick Family, Estate of Elisabeth Sloan Livingston, Universidad Nacional Autonoma de Mexico, 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. The School submitted a competitive renewal of this award in September 2011.

Student Achievements

The WSBS students continue to impress us all with their accomplishments. They published their research findings in prestigious international journals—more than 190 to date—and obtained fellowships to pursue their research interests. In addition, our current students and alumni (listed below) have been successful in receiving prestigious awards and fellowships.

- WSBS student Maria Joaquina Delas Vives was awarded a La Caixa Fellowship.
- WSBS student Elizabeth Nakasone was selected to attend the AACR's Translational Cancer Research for Basic Scientists Workshop.
- WSBS graduate Oliver Tam was awarded a Long-Term Fellowship from the Human Frontier Science Program.
- WSBS graduate Christopher Harvey received a Burroughs-Wellcome Fund Career Award at the Scientific Interface.

WSBS GRADUATES IN FACULTY AND SENIOR POSITIONS

Name	Current position
François Bolduc	Assistant Professor, University of Alberta, Canada
Darren Burgess	Assistant Editor, *Nature Reviews Cancer* and *Nature Reviews Genetics*, UK
Amy Caudy	Assistant Professor, University of Toronto, Canada
Catherine Cormier	Scientific Liaison, Arizona State University
Yaniv Erlich	Whitehead Fellow, Whitehead Institute
Rebecca Ewald	Project Manager, Roche Diagnostics, Inc.
Elena Ezhkova	Assistant Professor, Mount Sinai School of Medicine
Ira Hall	Assistant Professor, University of Virginia School of Medicine
Christopher Harvey	Assistant Professor, Harvard University
Keisha John	Associate Director, Recruitment and Undergraduate Research, WSBS
Zachary Lippman	Assistant Professor, Cold Spring Harbor Laboratory
Marco Mangone	Assistant Professor, Arizona State University
Patrick Paddison	Assistant Member, Fred Hutchinson Cancer Research Center
Emiliano Rial-Verde	Engagement Manager, McKinsey & Co., Inc., Geneva, Switzerland
Ji-Joon Song	Assistant Professor, Korea Advanced Institute of Science and Technology (KAIST), South Korea
Niraj Tolia	Assistant Professor, School of Medicine at Washington University, St. Louis
Wei Wei	Assistant Professor, University of Chicago

2011 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS

Calarco JP, Martienssen RA. 2011. Genome reprogramming and small interfering RNA in the *Arabidopsis* germline. *Curr Opin Genet Dev* **21:** 134–139. Review.

Chien Y,* **Scuoppo C**,* Wang X, Fang X, Balgley B, Bolden JE, Premsrirut P, Luo W, Chicas A, Lee CS, Kogan SC, Lowe SW. 2011. Control of the senescence-associated secretory phenotype by NF-κB promotes senescence and enhances chemosensitivity. *Genes Dev* **25:** 2125–2136.

Clark MB, Amaral PP, **Schlesinger FJ**, Dinger ME, Taft RJ, Rinn JL, Ponting CP, Stadler PF, Morris KV, Morillon A, Rozowsky JS, Gerstein MB, Wahlestedt C, Hayashizaki Y, Carninci P, Gingeras TR, Mattick JS. 2011. The reality of pervasive transcription. *PLoS Biol* **9:** e1000625. Review.

Fellmann C, Zuber J, **McJunkin K**, Chang K, **Malone CD**, Dickins RA, Xu Q, Hengartner MO, Elledge SJ, Hannon GJ, Lowe SW. 2011. Functional identification of optimized RNAi triggers using a massively parallel sensor assay. *Mol Cell* **41:** 733–746.

Honegger KS, Campbell RA, Turner GC. 2011. Cellular-resolution population imaging reveals robust sparse coding in the *Drosophila* mushroom body. *J Neurosci* **31:** 11772–11785.

Jiang L,* **Schlesinger F**,* Davis CA, ZhangY, Li R, Salit M, Gingeras TR, Oliver B. 2011. Synthetic spike-in standards for RNA-seq experiments. *Genome Res* **21:** 1543–1551.

Kopec CD, Bowers AC, **Pai S**, Brody CD. 2011. Semi-automated atlas-based analysis of brain histological sections. *J Neurosci Methods* **196:** 12–19.

Leung A, Cajigas I, Jia P, **Ezhkova E**, Brickner JH, Zhao Z, Geng F, Tansey WP. 2011. Histone H2B ubiquitylation and H3 lysine 4 methylation prevent ectopic silencing of euchromatic loci important for the cellular response to heat. *Mol Biol Cell* **22:** 2741–2753.

McJunkin K, Mazurek A, Premsrirut PK, Zuber J, Dow LE, Simon J, Stillman B, Lowe SW. 2011. Reversible suppression of an essential gene in adult mice using transgenic RNA interference. *Proc Natl Acad Sci* **17:** 7113–7118.

Pai S, Erlich JC, Kopec C, Brody C. 2011. Minimal impairment in a rat model of duration discrimination following excitotoxic lesions of primary auditory and prefrontal cortices. *Front Syst Neurosci* **5:** 74.

Premsrirut PK, Dow LE, Kim SY, Camiolo M, **Malone CD**, Miething C, **Scuoppo C**, Zuber J, Dickins RA, Kogan SC, Shroyer KR, Sordella R., Hannon GJ, Lowe SW. 2011. A rapid and scalable system for studying gene function in mice using conditional RNA interference. *Cell* **145:** 145–158.

Rangan P, **Malone CD**, Navarro C, Newbold SP, Hayes PS, Sachidanandam R, Hannon GJ, Lehmann R. 2011. piRNA production requires heterochromatin formation in *Drosophila. Curr Biol* **21:** 1373–1379.

Simpson DR, Yu M, Zheng S, Zhao Z, Muthuswamy SK, Tansey WP. 2011. Epithelial cell organization suppresses *myc* function by attenuating myc expression. *Cancer Res* **71:** 3822–3830.

Wasik KA, Rebbeck CA. 2011. Keystone symposia 40th season: microRNAs and noncoding RNAs in cancer. *Cancer Res* **71:** 6102–6105. Review.

Zamparini AL, Davis MY, **Malone CD**, Vieira E, Zavadil J, Sachidanandam R, Hannon GJ, Lehmann R. 2011. Vreteno, a gonad-specific protein, is essential for germline development and primary piRNA biogenesis in *Drosophila. Development* **138:** 4039–4050.

Zaratiegui M, **Castel SE**, Irvine DV, Kloc A, Ren J, Li F, de Castro E, Marín L, Chang AY, Goto D, Cande WZ, Antequera F, Arcangioli B, Martienssen RA. 2011. RNAi promotes heterochromatic silencing through replication-coupled release of RNA Pol II. *Nature* **479:** 135–138.

Zuber J,* **Rappaport AR**,* Luo W, Wang E, Chen C, Vaseva AV, Shi J, **Weissmueller S**, Taylor MJ, Weissenboeck M, Graeber TG, Kogan SC, Vakoc CR, Lowe SW. 2011. An integrated approach to dissecting oncogene addiction implicates a Myb-coordinated self-renewal program as essential for leukemia maintenance. *Genes Dev* **25:** 1628–1640.

Zuber J, Shi J, Wang E, **Rappaport AR**, Herrmann H, Sison EA, Magoon D, Qi J, Blatt K, Wunderlich M, Taylor MJ, Johns C, Chicas A, Mulloy JC, Kogan SC, Brown P, Valent P, Bradner JE, Lowe SW, Vakoc CR. 2011. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. *Nature* **478:** 524–528.

*These authors contributed equally to the work.
Watson School student.

- Former URP John Calarco was awarded the prestigious Harold M. Weintraub International Award for Graduate Studies. He was also named a "Top 30 under 30" by Forbes Magazine.
- WSBS graduate Elizabeth Murchison was one of *Genome Technology* Magazine's Young Investigators of the Year for 2011 for her research on "Cancer in the Wilderness." She also gave a lecture at the TEDGlobal conference in Edinburgh in 2011.

Mentoring Award for Greg Hannon

It was an honor to nominate my colleague, Gregory Hannon, for the prestigious 2011 Geoffrey Marshall Mentoring Award, which he received in April from the Northeastern Association of Graduate Schools. The award recognizes a mentor's outstanding support of graduate students from course completion through research and placement. The achievements of Greg's trainees have been uniformly outstanding. Four of his students completed their Ph.D. degrees in less than 4 years and two were recipients of the prestigious Harold M. Weintraub Graduate Student Award.

Alumni in Faculty and Senior Positions

To date, 49 students have received their Ph.D. degree from the WSBS. Of these graduates, 11 currently hold tenure-track faculty positions and two hold independent research positions. Twenty-seven of our former students are pursuing postdoctoral research and seven of our alumni completed their postdoctoral studies and have moved on to positions in administration, education, industry, management consulting, and journal editing. We are so proud of them all!

WSBS Family Events

Finally, I am pleased to announce that this was a great year for the WSBS students and administration as we celebrated some wonderful personal occasions. On January 30, just as the first recruitment weekend of 2011 was ending, Dawn Pologruto and her husband Tom welcomed a son, John Robert. Three students from the Entering Class of 2007 were married this year. On May 21, Maria Luisa Pineda Montoya married her fiancé Alvaro Echeverri Monsalve in Cartagena de Indias, Colombia.

John Robert Pologruto

Alvaro and Maria

On July 27, Eugene Plavskin married Alexandra Tsybeskov, and on September 21, Marek Kudla married Manuela Trzebinska. Also in July, Ozlem Mert, from the Entering Class of 2007, married Cem Aksoy in their native Turkey. On July 1, Eyal Gruntman, from the Entering Class of 2006, and his wife Neomi, along with big brothers Jonathan and Eli, welcomed daughter Ella to their family. Our congratulations and best wishes to all of them.

Leemor Joshua-Tor
Professor and Dean

Alexandra and Eugene

Manuela and Marek

Ozlem and Cem

Ella Gruntman

SPRING CURRICULUM

Topics in Biology

ARRANGED BY **Leemor Joshua-Tor, 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 CSHL faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the CSHL Lecture Courses held each summer at the Banbury Conference Center. In spring 2011, there were two such courses: Physical biology of the cell and Immunology.

Physical Biology of the Cell

Attended by the entering classes of 2007 and 2008

INSTRUCTOR **Rob Phillips,** California Institute of Technology

VISITING LECTURERS **Daniel Fisher,** Stanford University
Hernan Garcia, Princeton University
Thomas Gregor, Princeton University
Greg Huber, University of Connecticut
Justin Kinney, Cold Spring Harbor Laboratory
William Klug, University of California, Los Angeles
Jané Kondev, Brandeis University
Sanjoy Mahajan, Franklin W. Olin College of Engineering

TEACHING FELLOW **James Boedicker,** California Institute of Technology

The aim of this course was to provide a hands-on experience in the use of quantitative models as a way to view biological problems. The instructors began with "order of magnitude biology," showing how simple estimates can be exploited in biology. They demonstrated how to construct simple models of a variety of different biological problems, primarily using the tools of statistical mechanics. One of the key themes of the course was to show how physical biology unites and organizes topics in a fundamentally different way, often revealing that topics that are nearby in physical biology seem unrelated when viewed from the vantage point of molecular or cell biology. The students were guided from start to finish on several modeling case studies. The course ran from Saturday, March 6 to Friday, March 11.

Physical Biology of the Cell course participants: (*Left to right*) Thomas Gregor, Jané Kondev, Sanjoy Mahajan, Hernan Garcia, Leemor Joshua-Tor, James Boedicker, Nilgun Tasdemir, Ashutosh Agrawal, Ralph Burgess, Zhenxun Wang, Eugene Plavskin, Josh Sanders, Paloma Guzzardo, Maria Pineda, Susann Weissmueller, Michael Pautler, Kaja Wasik, Ozlem Mert, Joe Calarco, Beth Nakason, Zina Perova, Dario Bressan, Kyle Honegger, Justin Kinney, Bill Klug, Mitchell Bekritsky, Rob Phillips, Carrie Clendaniel, Philippe Batut, Elvin Garcia, Katie Liberatore, Marek Kudla, Greg Huber, Sang-Geol Koh

Immunology

Attended by the entering classes of 2009 and 2010

INSTRUCTOR	**Hidde Ploegh,** Whitehead Institute
GUEST LECTURER	**Juan Lafaille,** New York University
TEACHING FELLOWS	**Kevin Bohnam,** Harvard University **Deepali Malhotra,** Harvard University

Immunology is an interdisciplinary field that focuses on understanding the mechanisms by which multicellular organisms defend themselves against external threats of microbial aggression and internal threats associated with genetic instability and cellular transformation. The course focused on the unique elements of the innate immune system and the adaptive immune system. Innate immunity defends against microbes by recognizing evolutionarily conserved molecular patterns. The adaptive immune system has enormous flexibility in molecular recognition, but can also target self to cause autoimmune diseases. The course ran from Sunday to Saturday, March 27–April 2.

Immunology course participants: (*Left to right*) Lisa Krug, Nilgun Tasdemir, Wee Siong Goh, Nihan Kara, Arkarup Bandyopadhyay, Hidde Ploegh, Kevin Bonham (*kneeling*), Kristen Delevich, Colleen Carlston, Kaja Wasik, Beth Nakasone, Ozlem Mert, Ian Peikon, Cinthya Zepeda-Mendoza, Felix Schlesinger, Deepali Malhotra, John Sheppard, Juan Lafaille, Justin Kinney, Jack Walleshauser, Matthew Koh

Teaching Experience at the DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Amanda McBrien**, **Elna Gottlieb**, **Jennifer Galasso (Lead)**, **Erin McKechnie**, **Jermel Watkins**

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 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 DNA Learning Center instructors taught the Watson School students the didactic process—it was not until the fifth week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty the opportunity to get to know each other and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, in addition to the research mentors, the instructors of the Scientific Exposition and Ethics core course and members of the School's Executive Committee attend the talks and give individual feedback to students on their presentations. This year, 15 WSBS faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS **Florin Albeanu**, **Mickey Atwal**, **Anne Churchland**, **Josh Dubnau**, **Hiro Furukawa**, **Christopher Hammell**, **Gregory Hannon**, **Z. Josh Huang**, **Leemor Joshua-Tor**, **Adam Kepecs**, **Zachary Lippman**, **Stephen Shea**, **Nicholas Tonks**, **Glenn Turner**, **Anthony Zador**

FALL CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

INSTRUCTORS	**Gregory Hannon (Lead)**	**Bo Li**
	Alexander Gann	**Zachary Lippman**
	Leemor Joshua-Tor	**Nicholas Tonks**
GUEST LECTURERS	**Josh Dubnau**	**Robert Martienssen**
	Hiro Furukawa	**Senthil Muthuswamy**
	Christopher Hammell	**Christopher Vakoc**
	Alexei Koulakov	**Linda Van Aelst**

The SRL core course was revamped last year with regard to content and instructor participation. This year, only one module changed, with Bo Li joining the team. Each module of the course has been designed to cover a different general theme and proceeds with the goal of considering an open, still unanswered, scientific question. In addition, the first two modules of the course were combined into one longer module so that an integrated theme could be tackled using two different approaches: molecular biology and structural biology.

A fundamental aspect of earning the Ph.D. degree is training in the pursuit of knowledge. In this core course, which forms the heart of the curriculum, students (1) acquire a broad base of knowledge about the biological sciences, (2) learn the scientific method, and (3) learn how to think critically. The beginning of the course is divided into four to five modules, each of which has a different general theme, and proceeds with the goal of considering an open, still unanswered, scientific question. For each module, students read an assigned set of research articles (generally four articles), and at the end of the module, they provide written answers to a problem set that guides them through several of the articles.

Twice weekly, students attend lectures related to the module's topic that included concepts and fundamental information, as well as experimental methods. During each week, the students meet among themselves to discuss the assigned papers not covered by the problem set. Each week, students also spent an evening discussing the assigned articles with faculty. In the final part of the course, students participated in a mock study section in which real National Institutes of Health, R01 grants were reviewed and critiqued. This allowed the students to evaluate the questions before the answers are known, evaluate routes toward discovery before knowing where they will end, and make critical judgments about how to proceed in the face of an uncertain outcome.

The module topics for this course were as follows:

Topic	**Instructor(s)**
Mechanism and Structure of Gene Regulation	Alex Gann Leemor Joshua-Tor
The Brain: Wiring, Plasticity, and Maladaptation	Bo Li
Developmental Genetics and Morphological Diversity	Zachary Lippman
Signal Transduction and the Search for Novel Therapeutics	Nicholas Tonks
Study Section	Gregory Hannon

The Darrell Core Course on Scientific Exposition and Ethics

INSTRUCTORS	**David J. Stewart (Lead)** **Alea A. Mills** **Arne Stenlund**
TEACHING ASSISTANTS	**Christina Chen** **Aman Husbands**
GUEST LECTURERS	**Diane Esposito** **Jan A. Witkowski**
VISITING LECTURERS	**Olga Akselrod,** The Innocence Project **Keith Baggerly,** University of Texas, M.D. Anderson Cancer Center **Michael Beilski,** The Center for Biotechnology **Robert Charrow,** Greenberg Traurig, LLC **Avner Hershlag,** North Shore University Hospital **Amy Harmon,** *The New York Times* **Boyana Konforti,** Editor, *Nature Structural & Molecular Biology* **Tung-Tien (Henry) Sun,** New York University

The Scientific Exposition and Ethics (SEE) core course 2011 was taught by the same instructor team as last year, with David Stewart taking the lead role, joined by Alea Mills and Arne Stenlund. This year, two CSHL postdoctoral fellows, Christina Chen and Aman Husbands, participated in the course as teaching assistants. As usual, the course hosted expert guest lecturers who covered topics including scientific presentations, the editorial review process, the ethical and legal responsibilities of scientists, DNA profiling and postconviction appeals, and reproductive genetics.

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 for 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 integral aspects 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 are listed below:

Florin Albeanu
Gurinder (Mickey) Atwal
Anne Churchland
Josh Dubnau
Mikala Egeblad
Grigori Enikolopov
Hiro Furukawa
Thomas Gingeras
Christopher Hammell
Gregory Hannon
Z. Josh Huang
David Jackson
Leemor Joshua-Tor
Adam Kepecs
Alexei Koulakov
Adrian R. Krainer
Alexander Krasnitz
Bo Li
Zachary Lippman
Robert Martienssen
W. Richard McCombie
Alea A. Mills
Partha Mitra
Pavel Osten
Darryl Pappin
Michael Schatz
Stephen Shea
Raffaella Sordella
David L. Spector
Arne Stenlund
Bruce Stillman
Marja Timmermans
Nicholas Tonks
Lloyd Trotman
Glenn Turner
Christopher Vakoc
Linda Van Aelst
Doreen Ware
Michael Wigler
Anthony Zador
Hongwu Zheng
Yi Zhong

Specialized Disciplines Courses

In 2010, the School developed two new courses in the areas of statistics, programming, bioinformatics, and theoretical modeling and analysis. One of these courses, the Specialized Disciplines course in *Quantitative Biology*, was developed by Mickey Atwal and cotaught by Michael Schatz to provide students with the tools to interpret large, complex data sets and to use statistical methodology in their research. With the addition of this course, the students in the entering class of 2011 took a total of four Specialized Disciplines courses this fall. The other three Specialized Disciplines courses are *Cellular Structure and Function, Genetics and Genomics*, and *Systems Neuroscience*. *Cellular Structure and Function* was again taught by lead instructor Linda van Aelst and Raffaella Sordella, but it ran as a week-long intensive course when the students first arrived. Josh Dubnau led *Genetics and Genomics*, and he was joined again by Thomas Gingeras. Anthony Zador, Adam Kepecs, and Glenn Turner taught the *Systems Neuroscience* course.

Cellular Structure and Function

INSTRUCTORS	**Linda Van Aelst (Lead)** **Raffaella Sordella**
GUEST LECTURERS	**Darryl Pappin** **David L. Spector** **Bruce Stillman**
VISITING LECTURERS	**Aron Jaffe,** Novartis **Alexey Khodjakov,** Wadsworth Laboratory **Sandy Simon,** The Rockefeller University **Marc Symons,** The Feinstein Institute for Medical Research

With the complete genome sequence available for many organisms, there is now an increasing emphasis on understanding the function of the gene products. This understanding requires an increasing appreciation of the structure and function of the cell as well as 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.

Genetics and Genomics

INSTRUCTORS	**Josh Dubnau (Lead)** **Thomas Gingeras**
GUEST LECTURERS	**Christopher Hammell** **Marja Timmermans**

This course placed modern human genetics and genomics into the context of classical organismal genetics. History, perspective, and technique were described around four levels of analysis: naturally occurring variation, genome evolution, genetic screens, and gene epistasis. How do gene mutations help to define biological processes? How are more complex traits genetically dissected into simpler (underlying) components? What concepts and techniques are used to organize genes into pathways and networks? How are genes mapped, cloned, and engineered to identify functional domains of proteins? What gene variation exists in natural populations? What are the functional consequences of gene variation? How is it detected? How are genomes organized and coordinately regulated? How can genomic information be cataloged, organized, and mined? These questions and concepts were fleshed out using examples from the literature.

Quantitative Biology

INSTRUCTORS	**Mickey Atwal (Lead)** **Michael Schatz**
GUEST INSTRUCTORS	**Justin Kinney (CSHL Fellow)** **Alexander Krasnitz**

With the advent of high-throughput technologies in biology, it has become necessary for biological researchers to be able to analyze and interpret high-dimensional data and frame hypotheses mathematically. To this end, this course aims to equip the students with basic training in modern statistical methods. It is hoped that by the end of the course the students will be able to not only answer many of the simple statistical questions that arise in data analyses, but also be familiar with the more complex techniques used by fellow computational biologists. Topics to be covered include error fluctuations, calculating the significance of an experimental result, Bayesian inference, information theory, power calculations, dimensional reduction, and DNA sequence analyses. In addition, this course will introduce mathematical modeling, and this will be motivated by the classic examples in quantitative biology such as the Delbruck–Luria experiment, Hopfield's kinetic proofreading, and Kimura's neutral theory of population genetics.

Systems Neuroscience

INSTRUCTORS **Anthony Zador (Lead)**
Adam Kepecs
Glenn Turner

GUEST INSTRUCTORS **Florin Albeanu**
Anne Churchland
Alexei Koulakov
Stephen Shea

This course provided an overview of key aspects of neuroscience. The emphasis was on spanning levels: How can we go from molecules through cells and circuits to behavior? The three main components of the class are lectures, problem sets, and a final project in which students identify and present a neuroscience paper that spans levels, for example, from the molecular to the synaptic or the circuit level to the behavioral.

Watson students Maria Joaquina Delas Vives and Fred Marbach

POSTDOCTORAL PROGRAM

PROGRAM DIRECTOR **Nicholas Tonks**

PROGRAM ADMINISTRATOR **Alyson Kass-Eisler**

Cold Spring Harbor Laboratory (CSHL) is proud of its rich tradition in postdoctoral education and has a deep commitment to the postdoctoral experience. CSHL has long been recognized as a place for nurturing young scientists, with postdoctoral researchers being an integral part of the discovery process. 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's 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 day-to-day support for the postdoctoral program, which is overseen by the Dean of the Watson School, Dr. Leemor Joshua-Tor.

A New Postdoctoral Liaison Committee

In January 2011, Bruce Stillman held his annual Town Hall meeting with postdocs. One suggestion that came from the meeting was the establishment of a Postdoctoral Liaison Committee (PDLC), an elected group of postdocs who would help share information and ideas between the administration and the postdoctoral community. The six members elected to serve the first 1-year term on the PDLC are Kate Creasey, Santiago Jaramillo, Keerthi Krishnan, Dawid Nowak, Kate Revill, and Milos Tanurdzic. The PDLC has been hard at work. Its members have completed two informative surveys of issues affecting postdocs, which they have shared with the administration, and are busy organizing a postdoctoral retreat. The PDLC also submitted a response to the Biomedical Workforce Committee, a group established by the National Institutes of Health (NIH) Director to examine the future of the biomedical research workforce in the United States, on behalf of the CSHL postdocs.

Career Development Programs

The postdocs themselves have been very active in organizing career development lectures and workshops this year. A group of postdocs known as the IDP (or Individual Development Plan) group organized a five-part series called "Conversations with Faculty." In this series, CSHL faculty and administration gave the postdocs career insights in an informal and interpersonal format. The lecturers included Anne Churchland and Hongwu Zheng, two new faculty members who shared their experiences in applying for and getting an academic position; Stephen Shea and Glenn Turner, who talked about the challenges and joys of making the postdoc-to-faculty transition, setting up a lab, and surviving the first year as an Assistant Professor; Raffaella Sordella and Tony Zador, who spoke as senior faculty members and shared their insights from being members of faculty search committees; Sydney Gary and Charla Lambert, who discussed careers for life science Ph.D.s outside of ac-

ademia (science education and science administration); and Vlad Drozdoff and Darryl Pappin, who talked about technology transfer and intellectual property law careers.

To assist postdocs with the common "What do you do?" question, on March 14, the Watson School hosted Dr. Bob Tillman, Director of Faculty Development at Columbia University Medical Center, who gave a seminar on "Short talks that make big impressions: Promoting yourself and your science informally."

The IDP group also hosted an extremely well-received workshop on preparing for the Chalk Talk—an integral part of the academic job search. In the workshop, senior postdoctoral fellow Agustin Chicas presented his Chalk Talk to the group, which was moderated by faculty members Adrian Krainer, David Spector, and Nicholas Tonks.

The Bioscience Enterprise Club

The Bioscience Enterprise Club (BEC)—postdocs and students most interested in nonacademic careers—has recently formed. Members are in the process of organizing a great lineup of speakers for 2012. Their first seminar took place on October 5 and featured Tom Ippolito and Michael Bielski from the Fundamentals of the Bioscience Industry Program (FOBIP). In addition to teaching the FOBIP, Tom Ippolito is the Vice President of Regulatory Affairs/QA for Chembio Diagnostics, and Michael Bielski is the Assistant Director for Science & Technology Commercialization at the Center for Biotechnology and practices Intellectual Property Law at the law firm of Mayer, Ross & Hagen. Tom and Mike discussed careers in Regulatory Affairs and Intellectual Property Law and also discussed the Fundamentals of the Bioscience Industry Program.

Webinars

Through the Lab's Human Resources department, the postdocs have had access to free jobseeker webinars hosted by the Higher Education Research Consortium. The 2011 webinars included "The campus visit," "Expanding your career networks," and "Targeting your cover letter for faculty and nonfaculty positions."

Merck Ambassador Program

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, Dr. Maarten Hoek, a postdoctoral fellow from Dr. Bruce Stillman's laboratory, was hired as a Research Scientist in 2007, and Dr. Zuo Zhang, a postdoctoral fellow from Dr. Adrian Krainer's laboratory, joined MRL as a Research Scientist in January 2009. Dr. Li visited the Laboratory again on May 24 to talk about what it is like to work in a pharmaceutical company. Jing also gave a talk on his current research titled, "Applying genomic platforms to generate unbiased responder and treatment hypotheses for oncology translational research" to give the fellows a feel for the type of research being done in industry.

In-House Symposium Poster Prize

Six years ago, the School initiated a prize for the best poster 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. Fifteen posters were presented for the postdoctoral prize, which was won by Tim Kees, a postdoctoral fellow in Mikala Egeblad's laboratory.

Teaching Opportunities at the Watson School

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 serve 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 (TAs). These two fellows became an integral part of the course by providing their expertise in discussions, editing students' writing work, and critiquing oral presentations. This format was highly successful and was used again in fall 2011. Aman Husbands, a postdoctoral fellow from Marja Timmerman's laboratory, and Cristina Chen, from Chris Hammell's laboratory, were this year's TAs.

Demystifying Science

A new program called "Demystifying Science" has enlisted postdoctoral fellows to bring their expertise and teaching interests to the educationally diverse staff of the Laboratory. In these sessions geared toward a general audience, postdocs have talked about "Why haven't we cured cancer yet?" presented by Emma Vernersson-Lindahl; "Sensing the world: Insights from neuroscience" by Katharine Borges; "How plants defend themselves from viruses" by Aman Husbands; "Demystifying desire: How do we study the brain?" by Keerthi Krishnan; "Fighting cancer with tiny tools: Research from the Lowe lab" by Kiki Liu; and "How do we see the invisible?: Research from the Joshua-Tor lab" by Jon Ipsaro.

Professor for a Day

Another new program, in which postdocs have a major role, was designed by the CSHL Meetings and Courses program to communicate science to the broader public. "Professor for a Day" shows area high school students the inside story on scientific meetings and poster sessions. Postdocs have also contributed to the CSHL Meetings and Courses newsletter by writing short summaries of meetings or courses they have attended on campus.

New York Academy of Science: Science Alliance

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), 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 metro 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. This year, the Science Alliance meetings and workshops included "From idea to IPO: The technology venture course," "Thinking about teaching: Myths and realities of becoming an educator," "Bargaining for success: Mastering the science of negotiation," "Leadership and personal success through self-awareness and emotional intelligence," "From scientist to CSO: A business and industry prep course," and "Making the leap: A nonacademic career planning and job search boot camp." In addition, the biennial "What can you be with a Ph.D.?" was held on November 5th and 6th at New York University. Supported by a New York City regional consortium of colleges and universities, including CSHL, this 2-day preeminent career convention features exhibitors, industry representatives, symposia on options, and trends in career paths.

Scientific Societies

Scientific societies play a large part in shaping science, as well as the career development of its members. Lloyd Trotman, a CSHL faculty member and a member of the American Society for Cell Biology (ASCB), held a roundtable discussion on behalf of the ASCB to provide feedback from junior faculty and postdocs about how they think about and what they want from the Society. A similar session was led by CSHL postdoc Christos Noustos, an Ambassador to the American Society for Plant Biologists.

Cancer Gene Discovery Training Program

In 2011, the Laboratory received a training grant from the NIH. The primary goal of the Cancer Gene Discovery and Cancer Biology Postdoctoral Training Program is to prepare the next generation of cancer biologists to pursue independent research that integrates cancer gene discovery with functional biology to facilitate the development of novel cancer therapeutic, diagnostic, and/or preventive strategies.

Responsible Conduct of Research Course

An Ethics Course, which provided training in the Responsible Conduct of Research, was held this spring. The three-part course included sessions on "Use of biohazardous materials," "Human subjects and materials," "The ethical use of animals in research," "Roles and responsibilities associated with research funding," "Financial conflict of interest," and "Whistleblower policies," as well as a moderated presentation of the interactive video from the NIH, Office of Scientific Integrity, titled, "The lab: Avoiding research misconduct."

Grant Writing Workshop

On December 6th and 7th, the Office of Sponsored Programs, the Development Department, and the Postdoc Program Office held a very popular course on Grant Writing. The sessions included "Introduction to grants and external funding," "The grant application," "Protecting human research subjects/writing an IACUC," "The grant abstract/public summary: Dos, don'ts, and examples," "Peer review panel discussion," "Effective writing skills," "Fellowships, career development awards, and resources for postdocs," and a "Fellowship discussion panel" featuring postdocs Olga Anczukow-Camarda, Jonathan Ipsaro, and Keerthi Krishnan, who all currently hold competitive individual fellowships.

Success!

Finally, a most important measure of our postdoctoral program's success is the ability of postdoctoral fellows to secure positions at the end of their training. In 2011, the Laboratory's departing postdoctoral fellows went on to positions at the Institute of Molecular Pathology (IMP), Austria; Nanyang Technological University, Singapore; Novartis and Rutgers University, both in New Jersey; the University of Queensland, Australia; and the University of Toronto, Canada.

UNDERGRADUATE RESEARCH PROGRAM

PROGRAM DIRECTOR **Zachary Lippman**

PROGRAM ADMINISTRATOR **Keisha John**

Established more than 50 years ago, the CSHL Undergraduate Research Program (URP) plays a major part in providing and setting the standard for meaningful hands-on undergraduate research training in biology. The ten-week program begins the first week of June. During the first week, the students are oriented to the campus, and they receive a guided historical tour of campus and a tour of all facilities and resources available to them, ensuring a smooth transition into the Laboratory community and research. The URPs work, live, eat, and play among CSHL scientists, and they have a very busy academic and social calendar throughout the remaining 9 weeks of the summer. The students receive training in Scientific Research, Science Communication, Career Preparation, Bioinformatics, and Computational Biology, all while interacting socially with fellow program participants and members of the CSHL community at large in formal and informal activities. Some of these activities in 2011 were a pizza dinner with Dr. and Mrs. Watson, dinner with Dr. and Mrs. Stillman, BBQ and pool parties, volleyball games, designing the URP T-shirt, competing in the annual Scavenger Hunt, and the ever-famous URP vs. PI volleyball match.

The students' scientific development is the most important component of the program. Therefore, in the beginning of the summer, the URPs write an abstract and present a talk on their proposed research. The URP participants work alongside scientists and become increasingly independent throughout the summer. Concluding the program in August, the URPs prepare a final report and

The URPs have dinner with Dr. Watson.

present their results in a 15-minute talk at the URP Symposium. As in previous years, the program directors and faculty mentors were highly impressed with the accomplishments of the URP students. The following 28 students, selected from 727 applicants, took part in the 2011 program:

Paul Baranay, University of Notre Dame
Advisor: **Dr. Michael Schatz**
Sponsor: University of Notre Dame Scholar
Metassembler: A secret weapon for winning Assemblathon 2.

Tumas Beinortas, Cambridge University, England
Advisor: **Dr. Lloyd Trotman**
Sponsor: Emanuel Ax Fellow
Characterization of signature gene expression in *Pten*-loss-associated senescence, PlCS.

Lital Chartarifsky, Hebrew University of Jerusalem, Israel
Advisor: **Dr. Adrian Krainer**
Sponsor: Burroughs Wellcome Fellow
The alternative splicing factor SRSF6: A proto-oncogene?

Sai Chen, Life Science, Peking University, China
Advisor: **Dr. Greg Hannon**
Sponsor: Dr. Gregory Hannon Fellow
Preexperiments for optimized sensor assay.

Zachary Collins, George Washington University
Advisor: **Dr. Partha Mitra**
Sponsor: Hazen Fellow
Alterations in GABAergic neuroanatomy in autism spectrum disorder mouse models.

Thomas Dowling, Georgetown University
Advisor: **Dr. Greg Hannon**
Sponsor: Steamboat Foundation Scholar
Improving the signal-to-noise ratio of HITS-CLIP.

Katharine Dusenbury, Williams College
Advisor: **Dr. Darryl Pappin**
Sponsor: Dorcus Cummings Scholar
In vitro translation and mutational modification of *Grifola frondosa* metalloendopeptidase.

Claire Edgcumbe, University of British Columbia, Canada
Advisor: **Dr. Bo Li**
Sponsor: William Shakespeare Fellow
ErbB4's effect on the morphology of somatostatin cell in the thalamic reticular nucleus.

Thomas Erskine, Florida State University
Advisor: **Dr. Alexi Koulakov**
Sponsor: 30th Anniversary URP Scholar
Modeling the human brain: A mathematical approach.

Gregory Fitzgerald, Queens College
Advisor: **Dr. Pavel Osten**
Sponsor: 30th Anniversary URP Scholar
Anterograde tracing of the infralimbic cortex in Ng3 R451C and wild-type mice.

Ann-Desdemonia Fowajuh, University of Maryland Eastern Shore
Advisor: **Dr. Grisha Enikolopov**
Sponsor: William Townsend Porter Foundation Scholar
The molecular mechanism of NO and its role in cilia function.

Hannah Gendelman, Amherst College
Advisor: **Dr. Glenn Turner**
Sponsor: Von Stade Fellow
Light as a remote controller of the proboscis extension response in *Drosophila*.

Victoria Hanna, University of California, Irvine
Advisor: **Dr. Marja Timermanns**
Sponsor: National Science Foundation Scholar
microRNA mobility.

Brittany Haugen, Florida Institute of Technology
Advisor: **Dr. Alea Mills**
Sponsor: National Science Foundation Scholar
Investigating p63 isoforms in mouse models mimicking EEC syndrome.

Valentina Ignatova, St. Petersburg State University, Russia
Advisor: **Dr. Alex Krasnitz**
Sponsor: Robert H.P. Olney Fellow
Knowledge-based derivation of markers and subtypes in cancer.

Allison Kolbe, Ohio Wesleyan University
Advisor: **Dr. David Jackson**
Sponsor: National Science Foundation Scholar
Determination of phyllotaxy in maize by redox regulation of transcription factors.

Andrew Lawson, Cambridge University, England
Advisor: **Dr. Leemor Joshua-Tor**
Sponsor: Former URP Fund Scholar
Role of PIWI proteins in planarian regeneration. The structure and function of human mitochondrial CCA-adding enzyme.

Mitchell Leibowitz, University of Virginia
Advisor: **Dr. Richard D. McCombie**
Sponsor: National Science Foundation Scholar
Third-generation sequencing as a high-throughput diagnostic tool.

Chengyu Liu, University of Wisconsin, Madison
Advisor: **Dr. Mickey Atwal**
Sponsor: Garfield Fellow
Cancer biomarkers investigation in human array CGH data in learning and memory through reward learning in *D. melanogaster.*

Monica Manglani, Lafayette College
Advisor: **Dr. Steve Shea**
Sponsor: Libby Fellow
Role of neuronal inhibition in vocal communication.

Kelly Mulfaul, Trinity College Dublin, Ireland
Advisor: **Dr. Bruce Stillman**
Sponsor: Trinity College Fellow
Role of CMG helicase in nucleosome disassembly.

Benjamin Perrella, Hunter College
Advisor: **Dr. Yi Zhong**
Sponsor: Hunter College Scholar
Role of the *NF1* gene.

Kristian Saied, University of Puerto Rico
Advisor: **Dr. Josh Dubnau**
Sponsor: H. Bentley Glass Fellow
Ago2 protein as the protector against R2 retrotransposons in *Drosophila* brain.

Sarah Shareef, Harvard University
Advisor: **Dr. Chris Vakoc**
Sponsor: Alfred L. Goldberg Fellow
SMARCA4: A potential therapeutic target for acute myeloid leukemia.

Burak Tepe, Bogazici University, Turkey
Advisor: **Dr. Adam Kepecs**
Sponsor: Joan Redmond Read Fellow
Role of cholinergic neurons in regulating attention.

Anne Turberfield, Cambridge University, England
Advisor: **Dr. Chris Hammell**
Sponsor: James D. Watson Fellow
Systematic RNAi screen to identify developmental regulators of microRNA activity.

Jeanette Wat, Rice University
Advisor: **Dr. Scott Powers**
Sponsor: James D. Watson Fellow
Oncogene dependency in HCC.

Kevin Wu, University of California, San Diego
Advisor: **Dr. Doreen Ware**
Sponsor: National Science Foundation Scholar
De novo transcriptome assembly and analysis of RNA-seq data from maize and sorghum in the cloud.

PARTNERS FOR THE FUTURE

PROGRAM DIRECTOR **David Jackson**

PROGRAM ADMINISTRATOR **Elizabeth Soldatic**

The Partners for the Future Program, established by Dr. James Watson in 1990, provides an opportunity for talented Long Island high school students to gain hands-on experience in biomedical research at Cold Spring Harbor Laboratory (CSHL). 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 15 top students are chosen to participate in the program. Students selected for 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 in a lab. Interacting with scientists and support staff, the students learn to define and pursue a research goal while solving problems that may occur along the way. The 2011–2012 Partners for the Future listed below were chosen from among 39 nominations:

Partner	High School	CSHL Mentor	Laboratory
Angad Anand	Cold Spring Harbor	Tony Zador	Santiago Jaramillo
Charles Bill	Cold Spring Harbor	Zach Lippman	Ke Jiang
Madison Bondy	South Side	Josh Huang	Jiangteng Lu
Beverly Chen	Syosset	Doreen Ware	Lifang Zhang
Sean Culkin	South Side	Marja Timmermans	Kate Petsch
Holly Flores	Huntington	Senthil Muthuswamy	Mike Feigen
Annalise Grammerstorf	Harborfields	Yi Zhong	Yichun Shuai
Andrew Ku	Huntington	Adam Kepecs	Hyun Jae Pi
Justin Levine	Friends Academy	James Hicks	Jim Hicks
Connie Long	Half Hollow Hills East	Adrian Krainer	Yimin Hua
Alexandra Marshall	North Shore	David Jackson	David Jackson
Japbani Nanda	W.T. Clarke	Robert Martienssen	Benjamin Roche
Joseph Nolan	Harborfields	Alea Mills	Assaf Vestin
Daniel Sikavi	Roslyn	Mikala Egeblad	Jae-Hyun Park
Billy Smith	Cold Spring Harbor	Adam Kepecs	Sachin Ranade

2011–2012 CSHL Partners for the Future: (*Seated, left to right*) Angad Anand, Annalise Grammerstorf, Connie Long, Justin Levine. (*Standing, left to right*) Holly Flores, Alexandra Marshall, Andrew Ku, Sean Culkin, Beverly Chen, Charles Bill, Daniel Sikavi, Madison Bondy, Joseph Nolan, Dr. David Jackson. (*Not pictured*) Japbani Nanda, Billy Smith

MEETINGS AND COURSES

ACADEMIC AFFAIRS

The Meetings and Courses program attracts scientists from all over the world to communicate, learn, and critique the latest ideas and approaches in the biological sciences. 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 9000 scientists ranging from graduate students and postdoctoral fellows to senior faculty come from 'round the world to attend these events at Cold Spring Harbor, and ~2700 scientists participated in our new program in Asia.

In 2011, the 30 laboratory and lecture courses that were held at the Laboratory attracted more than 1330 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.

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor. It is their excellence and dedication that make the course program work so well. We would especially like to thank Drs. Mark Alkema, Tracy Andacht, Heather Broihier, James Bruce, Vincent Carey, David Featherstone, Nace Golding, Eric Lambie, Andrew Link, Eric Miska, Matthew Nolan, Alison North, Samara Reck-Peterson, Jeffrey Strathern, Scott Thompson, Jim Tisdall, and John Tyson, who all 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 pro-

Lunch on the lawn

gram. 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 29 academic meetings this year, which brought together more than 7800 scientists worldwide to discuss their latest research. The Symposium this year focused on metabolism and disease, highlighting the emerging integration of molecular biology with the classical biochemistry of energy utilization in the cell. The Symposium attracted almost 300 participants including notable biologists such as Mike Brown, Ron Evans, Joe Goldstein, Lenny Guarente, Cynthia Kenyon, Steven McKnight, David Sabatini, Bruce Spiegelman, Craig Thompson, Eileen White, and Douglas Wallace.

Cold Spring Harbor Laboratory meetings are unique in assembling a program with very few invited speakers. Instead, meeting organizers are encouraged to select talks from abstracts that are submitted on the basis of scientific merit, ensuring that the latest findings are presented and that young scientists have the chance to describe their work. The annual meetings on The Biology of Genomes, Retroviruses, and Neurobiology of *Drosophila* were all oversubscribed, and many others attracted strong or record attendances. New meetings were launched on Stem Cell Engineering and Cell-Based Therapies and The Biology of Cancer: Microenvironment, Metastasis, and Therapeutics, and the biennial Eukaryotic mRNA Processing meeting began with an earthquake and ended with hurricane Irene! 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 NIH, NSF, foundations, and companies. Core support for the meetings program is provided by the Laboratory's Corporate Sponsor Program.

The development of the Cold Spring Harbor Asia program, as a wholly owned affiliate of Cold Spring Harbor Laboratory in Suzhou, China, continued with 13 scientific meetings and one Summer School held throughout the year. The Cold Spring Harbor Asia Symposium focused on synthetic biology and highlighted exciting work from around the world, ongoing in this burgeoning field.

The Meetings and Courses program staff comprises a lean team of talented professionals, many of whom wear multiple "hats," who handle the complexities of database design, programming, web and multimedia design, educational grants management, marketing and recruitment, conference and course administration, audiovisual services, and other activities. Two new staff members, Dr. Charla Lambert and Brad Frey, joined the team in early 2011 to fill new positions as program manager, science and training, and marketing associate, respectively.

Terri Grodzicker
Dean
Academic Affairs

David Stewart
Executive Director
Meetings and Courses Program

76TH COLD SPRING HARBOR LABORATORY SYMPOSIUM ON QUANTITATIVE BIOLOGY

Metabolism and Disease

June 1–6 291 Participants

ARRANGED BY **Bruce Stillman, Terri Grodzicker, and David Stewart**
Cold Spring Harbor Laboratory

S. Mandrup, D. Stewart

Cold Spring Harbor Laboratory selected the theme of Metabolism and Disease for the historic 76th Symposium in the series. The decision to focus the 2011 Symposium on this topic reflects the growing convergence of lines of research in fields ranging from cancer biology to aging, demonstrating that key metabolic pathways regulate energy homeostasis in cells, organs, and whole organisms and that many human diseases share profound dysfunction in metabolism at a molecular level. Previous Symposia that have been devoted in all or in part to metabolism and its dysfunction include Biological Oxidations (1939), The Mammalian Fetus: Physiological Aspects of Development (1954), Cellular Regulatory Mechanisms (1961), The Cardiovascular System (2002), and Clocks and Rhythms (2007).

A key theme of early 20th-century investigation, cellular bioenergetics, yielded a vast body of biochemical knowledge about key energy transformation processes such as oxidative phosphorylation and glycosylation. In contrast, the molecular biology revolution of the last half century has focused largely on how cells and organisms process information stored in nucleic acid and how these mechanisms are regulated during fundamental processes such as cell division and development or adapt and respond to external conditions. Tremendous light has been shed on processes such as DNA replication, transcription, RNA processing, and protein translation, and more recently on signaling cascades, developmental lineages, and disease progression (e.g., oncogenesis). Much of this work has been done with little regard for the metabolic state of the cell, which could be safely ignored while the reductionist approach yielded such powerful results. But energy consumption at the cellular and organismal level is once again becoming a major theme of modern research, because imbalances in these processes are increasingly understood to be major contributory factors in many diseases that significantly impact society, including obesity, cardiovascular disease, diabetes, metabolic disorders, and cancer. Moreover, energy homeostasis appears to have a significant impact on the aging process itself.

The Symposium aimed to integrate a very broad field of investigative effort, bringing together advances in our understanding of energy intake, consumption, and storage (diet, exercise, and fat), oxygen regulation and hypoxia, circadian rhythms, and life span/aging. The Symposium explored metabolism at the molecular (gene expression, posttranslational modifications, protein turnover, cofactors and integrators, hormones, and signals), organellar (mitochondria), cellular, organ system (cardiovascular, bone), and organismal (timing and life span) scales. Diseases impacted by metabolic

B. Stillman, R. Evans, J. Goldstein

imbalance or dysregulation that were covered in detail included diabetes, obesity, metabolic syndromes, and cancer. New and emerging technologies were presented for simultaneous monitoring of hundreds of metabolites that allow for sophisticated sampling of the metabolic state of cells.

In arranging this Symposium, the organizers were dependent on the guidance of a broad cadre of advisors including Drs. Ron Evans, Lenny Guarente, David Sabatini, Bruce Spiegelman, Craig Thompson, Eileen White, and Douglas Wallace. Opening night speakers included Douglas Wallace, Ron Evans, Cynthia Kenyon, and Craig Thompson. Nobel laureates Joe Goldstein and Mike Brown jointly presented the Reginald Harris Lecture on "Surviving Starvation: Essential Role of the Ghrelin-Growth Hormone Axis." Cynthia Kenyon delivered a compelling Dorcas Cummings Lecture on "The Deadly Sweet Tooth" to Laboratory friends, neighbors, and Symposium participants in advance of the annual dinner parties. Steven McKnight concluded the meeting with a masterful and thought-provoking summary.

C. Kenyon, D. Mangelsdorf

This Symposium was attended by almost 300 scientists from more than 20 countries, and the program included 62 invited presentations and 131 poster presentations. To disseminate the latest results and discussion of the Symposium to a wider audience, attendees were able to share many of the Symposium talks with their colleagues who were unable to attend using the Leading Strand video archive, and interviews by Emilie Marcus, Nicola McCarthy, Miranda Robertson, Richard Sever, and Jan Witkowski with leading experts in the field were arranged during the Symposium and distributed as free video from the Cold Spring Harbor Symposium interviews website.

Funds to support this meeting were obtained from the National Institute of General Medical Sciences, the Eunice Kennedy Shriver National Institute of Child Health and Human Development, the National Institute of Diabetes and Digestive and Kidney Diseases, and the National Institute on Aging, all branches of the National Institutes of Health; Amgen; and Seahorse Biosciences. Financial support from the corporate sponsors 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*

Fat, Exercise, and Rhythms
Chairperson: B. Kahn, *Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts*

Insulin Resistance and Sensitivity
Chairperson: J. Goldstein, *University of Texas Southwestern Medical Center, Dallas*

Life Span, Aging, and Cancer
Chairperson: J. Brugge, *Harvard Medical School, Boston, Massachusetts*

Regulation and Dysregulation of Metabolism
Chairperson: D. Sabatini, *Whitehead Institute, Cambridge, Massachusetts*

Reginald B. Harris Lecture: Surviving Starvation: Essential Role of the Ghrelin–Growth Hormone Axis
M.S. Brown and J.L. Goldstein, *University of Texas Southwestern Medical Center, Dallas*

Signaling and Gene Regulation
Chairperson: D. Botstein, *Princeton University, New Jersey*

Environment and Sensing
Chairperson: K. Vousden, *Beatson Institute for Cancer Research, Glasgow, United Kingdom*

Dorcas Cummings Lecture: The Deadly Sweet Tooth
C. Kenyon, *University of California, San Francisco*

Metabolic Reprogramming
Chairperson: J. Yuan, *Harvard Medical School, Boston, Massachusetts*

Clocks and Intermediary Metabolism
Chairperson: S. McKnight, *University of Texas Southwestern Medical Center, Dallas*

Metabolism and Cancer
Chairperson: E. White, *Rutgers University, The Cancer Institute of New Jersey, New Brunswick*

Autophagy, Timing, and Small RNAs
Chairperson: T. Mak, *Ontario Cancer Institute, Toronto, Canada*

Summary
S. McKnight, *University of Texas Southwestern Medical Center*

Symposium picnic

N. Sonenberg, D. Spector

MEETINGS

Systems Biology: Networks

March 22–26 154 Participants

ARRANGED BY **Patrick Aloy,** Institute for Research in Biomedicine
Trey Ideker, University of California
Marian Walhout, University of Massachusetts Medical School

T. Ideker, P. Aloy, M. Walhout

Biological interaction networks have been in the scientific limelight for more than a decade. Increasingly, the concept of network biology and its various applications are becoming more commonplace in the community. Recent years have seen networks move from pretty pictures with limited application to solid concepts that are increasingly used to understand the fundamentals of biology. These networks span multiple scales of space and time, from networks of interacting atoms, which influence protein structure, to networks of genes and proteins with direct implication in complex disease processes. As in previous editions, this conference gathered together many of the leading minds in the world of cell networks and systems biology to summarize the most promising recent advances and discuss how future developments will unfold.

This year, we had a 5-day program organized into four main topics: gene regulatory networks, protein–protein interaction networks, signaling networks, and, for the first time, single-cell networks. The program opened with the first of three Keynote Addresses from Anne-Claude Gavin on the organization of the cell machinery of the small bug *Mycoplasma genitalium* and the application of affinity purification methods to chart systematically novel types of biological networks between proteins and small molecules, lipids, or metabolites. In the following days, the program continued with 20 invited presentations and 28 short talks selected from posters, all of them of outstanding quality, covering many aspects of network and systems biology, from the development of novel methodologies to chart interactions with spatiotemporal resolution to several biomedical applications. However, arguably, the best-received contributions, which also triggered very lively discussions, were those where the use of network biology shed light on key biological processes or questioned classical textbook knowledge. The accepted posters were divided into two groups and displayed on Wednesday and Thursday evenings, in a very relaxed atmosphere during wine and cheese mixers. On Friday evening, we had the pleasure to attend the second Keynote Address from James Collins, who gave us an exceptional overview of the current state of synthetic biology and discussed the key role that networks have in the development of models. The evening ended with the traditional lobster banquet and drinks. The full program concluded on Saturday with our third Keynote delivered by James Fowler and with a session devoted to signaling networks, with a particular emphasis on biomedical implications and applications, and a lively discussion on the future of network biology and how the meeting should evolve to incorporate novel developments.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.

PROGRAM

Keynote Address
A.-C. Gavin, *EMBL-Heidelberg*

Gene Regulatory Networks I
Chairperson: B. van Steensel, *Netherlands Cancer Institute, Amsterdam*

Single Cell Networks
Chairperson: A. Califano, *Columbia University, New York*

Protein–Protein Interaction Networks I
Chairperson: S. Michnick, *Université de Montréal, Canada*

Signaling Networks I
Chairperson: I. Stagljar, *University of Toronto, Canada*

Gene Regulatory Networks II
Chairperson: T. Hughes, *University of Toronto, Canada*

Protein–Protein Interaction Networks II
Chairperson: A. Keating, *Massachusetts Institute of Technology, Cambridge*

Keynote Address
J. Collins, *Boston University, Massachusetts*

Signaling Networks II
Chairperson: C. Myers, *University of Minnesota, Minneapolis*

Keynote Address
J. Fowler, *University of California, San Diego*

F. Holstege, O. Bahcall

A. Ergun, D. Furman

Computational Cell Biology

March 29–April 1 111 Participants

ARRANGED BY **Galit Lahav,** Harvard Medical School
Leslie Loew, University of Connecticut Health Center
Wallace Marshall, University of California, San Francisco
John Tyson, Virginia Polytechnic Institute and State University

The year 2011 was an exciting one for this meeting. The successful format of previous CSHL meetings was maintained, and three new sessions were added. Each session was chaired by an established leader in the field who gave a talk, followed by shorter talks chosen from abstracts. All chairs were actively involved in nominating abstracts for oral presentations. Many talks combined both experimental and computational work, showing the power and synergy between the two approaches and providing evidence that the field is going in the right direction. The quality of the poster session was also very high.

G. Lahav, S. Rafelski

The meeting opened with a Keynote Address by Tobias Meyer, who talked about the use of single-cell analysis and mathematical models to understand complex signaling systems such as chemotaxis and collective cell migration. His talk was followed by a new session—Beyond Imaging: Modeling and Inference from Microscopy Data—chaired by G. Danuser, who presented a unique approach for studying signal transductions through measurements of cell fluctuations in basal conditions and showed how it can shed light on even well-studied systems such as cytoskeleton regulation. D. Lauffenberger opened the second day with an exciting talk about the use of multivariant computational modeling to extract tissue level variation. The second session that morning was also new for this meeting—Noise and Homeostasis, chaired by J. Hasty, who presented new ideas on how to build genetic circuits to understand homeostatic control in cells under both static and oscillatory modes. Each of these sessions was followed by a number of short talks chosen from abstracts.

Denis Bray gave an inspirational second Keynote Address about the past and future of computational cell biology and the main challenges in the field. His talk was followed by a session on synthetic biology, which was again new for this meeting. It was chaired by P. Silver, who claimed that "biology is the technology of this century" and gave a stimulating talk about design and use of living devices to perform specific tasks such as carbon fixation.

The third day, J. Lederer presented results on modeling of calcium signaling in cardiac cells. E. Munro then addressed the interplay between small-scale biochemical activities of cytoskeletal proteins and large-scale mechanical properties and motility of whole cells. In the afternoon, we were treated to a session on New Tools and Software, chaired by I. Moraru. The afternoon poster session covered a broad range of exciting topics, ranging from modeling bacterial motility to the development of new computational architectures based on field programmable gate arrays for biological network simulations. One important theme that emerged in several posters is the growing emphasis on studying gene regulation in the frequency domain.

The Modeling Cells in Cancer and Development session was met with significant enthusiasm and contained several fascinating reports, including the use of extended time-lapse automated microscopy to quantitatively analyze quiescence in cells, the role of cell lineage and feedbacks in con-

trolling growth and rate of cell divisions of stem cells and tumors, and the use of developing lung as a model to study the connection between epithelial tube shape and cell division.

A special feature of this meeting was a lively improvisational workshop for scientists led by R. Holmes, who used improvisational theater exercises and games to allow people to get to know one another and build a social context for conversations across disciplines and academic positions.

The meeting was supported by a grant from the National Science Foundation (NSF) as well as additional contributions from various companies and will continue on its biennial schedule (March/April 2013). More money has to be applied for further meetings. NSF representative Richard Rodewald showed enthusiasm for continued support of the meetings and confirmed that the topic of this meeting is a priority for the NSF.

A. Shirinifard, E. Spanjaard

PROGRAM

Keynote Address: Local Signals at the Leading Edge Control Directional Migration
T. Meyer, *Stanford University, California*

Beyond Imaging: Modeling and Inference from Microscopy Data
Chairperson: G. Danuser, *Harvard Medical School, Boston, Massachusetts*

Signal Processing and Decision Making
Chairperson: D. Lauffenberger, *Massachusetts Institute of Technology, Cambridge*

Noise and Homeostasis
Chairperson: J. Hasty, *University of California, San Diego*

Keynote Address: Bacterial Chemotaxis and Beyond
D. Bray, *University of Cambridge, United Kingdom*

F. Naef, J. Tyson

Synthetic Biology
Chairperson: P. Silver, *Harvard Medical School, Boston, Massachusetts*

Cell Physiology
Chairperson: W. Jonathan Lederer, *University of Maryland, Baltimore*

Cell Mechanics: Shape, Motility, and Cytoskeleton
Chairperson: E. Munro, *University of Chicago, Illinois*

New Tools and Software
Chairperson: I. Moraru, *University of Connecticut Health Center, Farmington*

Improvisational Workshop for Scientists
Chairperson: R. Holmes, *University of Connecticut Health Center, Farmington*

Modeling Cells in Cancer and Development
Chairperson: V. Quaranta, *Vanderbilt University, Nashville, Tennessee*

Stem Cell Engineering and Cell-Based Therapies

April 7–10 109 Participants

ARRANGED BY **Rudolf Jaenisch,** Whitehead Institute/Massachusetts Institute of Technology
Michel Sadelain, Memorial Sloan-Kettering Cancer Center
Amy Wagers, Harvard University

D. Deyle, R. Jaenisch

The derivation of human pluripotent stem cells, their differentiation, and their genetic modification offer tantalizing prospects for future cell therapies and regenerative medicine. This meeting focused on recent advances in our understanding of pluripotency and cancer stem cells; cell differentiation into mesodermal, endodermal, and neural fates; and emerging technologies for safe and accurate genetic engineering of stem cells, myocardium, and T lymphocytes. Most of the presented research focused on human cells, albeit not exclusively, and included clinical trial presentations.

Irving Weissman opened the meeting by summarizing stem cell hierarchy in the hematopoietic system and its relationship to leukemia stem cells, extending the discussion to self-renewal and survival strategies found in malignant cell types. Jacob Hannah reported on the conversion between epiblastic and ground-state stem cells. Konrad Hochedlinger discussed the biology of Sox2 in normal cells, tumors, and somatic cell reprogramming.

G. Keller explained how early in vitro patterning conditions determine cell fate decisions between mesodermal lineages, and H. Mikkola pinpointed the interplay between hematopoietic and myocardial fates controlled by Scl/Tal1. D. Srivastava and K. Chien went on to present alternate approaches for generating cardiomyocytes, based on either *trans*-differentiation or directed differentiation from newly defined human progenitor cells.

I. Verma discussed the pros and cons of in vivo gene therapy versus potential stem-cell-based approaches for the treatment of liver diseases, a theme later addressed by D. Williams, who discussed the regulatory hurdles one must overcome to translate novel stem cell therapies based on his experience with severe combined immunodeficiency. F.H. Gage discussed the intriguing potential of induced pluripotent

R. Cottle, I. Fernandez

V. Sumramnian, J. Muffat

stem cells to tackle complex neurodevelopmental disorders and how to possibly investigate behavioral anomalies, and other speakers discussed progress toward cell-based therapies for Parkinson's disease.

Emerging technologies for stem cell engineering were covered in depth, with ample discussion of bacterial artificial chromosomes, targeted nucleases including zinc finger nucleases, homing endonucleases, and TALE nucleases, and adenoviral- and adeno-associated virus-mediated homologous recombination. E. Papapetrou articulated the concept of genomic safe harbors, and K. Chien and C. June presented work on RNA transfection to engineer cells avoiding DNA integration. Exciting clinical results utilizing genetically modified T cells to treat HIV/AIDS or leukemia were also presented by C. June, and R. Brentjens, and Y. Shi presented intriguing preliminary data on the use of mesenchymal stem cells to alleviate hepatic cirrhosis.

This meeting was funded in part by Fluidigm and Molecular Devices.

A.R. Migliaccio, Y. Shi

PROGRAM

Keynote Address: Normal and Neoplastic Stem Cells
I.L. Weissman, *Stanford University, California*

Pluripotent and Cancer Stem Cells
Chairperson: R. Jaenisch, *Whitehead Institute/Massachusetts Institute of Technololgy, Cambridge*

Differentiation I: Mesodermal Lineages
Chairperson: A. Wagers, *Harvard University, Cambridge, Massachusetts*

Differentiation II: Endodermal Lineages
Chairperson: I. Verma, *The Salk Institute, La Jolla, California*

Stem Cell Engineering
Chairperson: M. Sadelain, *Memorial Sloan-Kettering Cancer Center, New York*

Differentiation III: Neuroectodermal Lineages
Chairperson: P. Aubourg, *INSERM and University Paris Descartes, France*

Keynote Address: Studying the Cellular and Molecular Basis of Neurodevelopmental Disease with iPS Cells
F.H. Gage, *The Salk Institute for Biological Studies, La Jolla, California*

Immune Engineering and Mesenchymal Stem Cells
Chairperson: C. June, *University of Pennsylvania, Philadelphia*

Special Lecture: Driving Pluripotent Stem Cells toward a Ventriculogenic Fate: Epigenetic and Modifed RNA Pathways

D. Cohen, F. Stewart

H. Mikkola, G. Keller

Synapses: From Molecules to Circuits and Behavior

April 12–16 327 Participants

ARRANGED BY **Hollis Cline,** The Scripps Research Institute
Richard Huganir, Johns Hopkins University School of Medicine/HHMI
Thomas Sudhof, Stanford University School of Medicine/HHMI

R. Huganir, M. Scanziani

The brain consists of a vast network of excitable cells (neurons) that conduct electrical impulses and communicate with one another 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 system. The convergence of advances in biochemistry, molecular genetics, microscopic imaging, and electrophysiology has made synaptic biology one of the most exciting and rapidly growing fields 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.

R. Kaletsky, J. Schulke

J. Jakkamsetti, K. Spilker

This fourth meeting was very successful, bringing together nearly 200 participants from the United States, Europe, and Asia in an atmosphere of social and scientific exchange. Presenters discussed a wide range of topics emphasizing the basic physiology of synapses, neurons, and circuits as well as pathophysiological aspects of nervous system functions. More than 50 attendees were selected to give oral presentations of their work. The two poster sessions were sites of active discussion of data and concepts. Outstanding plenary lectures were given by Reinhard Jahn and Mark Bear.

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

Synapse Development
Chairpersons: A. Majewska, *University of Rochester, New York*; L. Mei, *Medical College of Georgia, Augusta*

Ion Channels, Receptors, and Transporters
Chairpersons: K. Roche, *NINDS/National Institutes of Health, Bethesda, Maryland;* E. Perozo, *University of Chicago, Illinois*

Keynote Address: Exocytosis of Synaptic Vesicles: A Molecular View
R. Jahn, *Max-Planck Institute for Biophysical Chemistry*

Synaptic Function and Plasticity
Chairpersons: B. Sabatini, *Harvard Medical School, Boston, Massachusetts;* P. Worley, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Optogenetics: From Molecules to Behavior
Chairpersons: A. Bonci, *National Institute on Drug Abuse, Baltimore, Maryland;* A. Kreitzer, *J. David Gladstone Institutes, University of California, San Francisco*

Imaging Neuronal Circuits
Chairpersons: J. Wang, *University of California, San Diego;* C. Gilbert, *The Rockefeller University, New York*

Keynote Address
M. Bear, *HHMI/Massachusetts Institute of Technology*

Neuronal and Circuit Plasticity
Chairpersons: S. du Lac, *The Salk Institute, La Jolla, California;* M. Scanziani, *University of California, San Diego*

Behavioral Plasticity
Chairpersons: J. LeDoux, *New York University, New York;* Y. Dudai, *Weizmann Institute of Science, Rehovot, Israel*

Diseases of Synapses and Circuits
Chairpersons: L. Monteggia, *University of Texas Southwestern Medical Center, Dallas;* H. Song, *Johns Hopkins University, Baltimore, Maryland*

W. Hung, M. Zhen

O. Steward, A.M. Craig

The Biology of Cancer: Microenvironment, Metastasis, and Therapeutics

April 26–30 340 Participants

ARRANGED BY **Senthil Muthuswamy,** Ontario Cancer Institute
Kornelia Polyak, Dana Farber Cancer Institute
David Tuveson, Cambridge Research Institute

Cancer is now recognized as an abnormal organ where multiple signals and cell types cooperate during cancer initiation and metastatic progression. Significant progress is being made on identifying and characterizing different cell types, understanding the mechanisms by which these cell types interact with one another, developing ways to image the changes in a tumor, and exploring therapeutic opportunities that take into account the non-cancer cells. Thus, there is an emergence of a need for an integrative approach for controlling cancer. This meeting brought together biochemists studying cell metabolism; physicists, developing ways to image cells; immunologists, developing ways to harness the immune system; cell biologists, identifying new ways to stop the growth of tumors; and mouse modelers, developing models to study human cancers. Including Keynote Speaker Charles Sawyers, several prominent cancer researchers who are pioneers and thought leaders in their fields presented and participated in the meeting. In addition, we held two panel discussions including "Will personalized cancer medicine (PCM) work? Will xenograft or GEM mouse models help us address the issue," and another on the topic of "Stemness versus plasticity: What drives and maintains cancer?" Both panel discussions were provocative and an effective means to communicate the expert views and elicit audience participation. The meeting, as a whole, was received very well and the participants enjoyed both the diversity and the depth of topics covered.


S. Muthuswamy

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health; Astellas; Applied BioPhysics; and Molecular Devices.

PROGRAM

Integrated Discovery Platforms and Personalized Medicine
Chairperson: D. Epstein, *OSI Pharmaceuticals, Farmingdale, New York*

Keynote Address
C. Sawyers, *HHMI/Memorial Sloan-Kettering Cancer Center*

Modeling Cancer in Mice: Xenograft and Genetically Engineered Models
Chairpersons: S. Lowe, *Cold Spring Harbor Laboratory, New York;* B. Neel, *Ontario Cancer Institute, Toronto, Canad*a

Panel Discussion/Debate: Will Personalized Cancer Medicine Work and **Will Xenograft or GEM Mouse Models Help Us Address the Issue?**
Moderators: D. Tuveson; D. Epstein
Panelists: C. Sawyers, B. Neel, G. Evans, L. Chin, S. Lowe, J. Jonkers, T. Mak, L. Coussens

Genetics and Biology of Tumor Cell Architecture
Chairpersons: I. Macara, *McGill University, Montreal, Canada;* V. Vasioukhin, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Tumor Heterogeneity and Cancer Cell Plasticity
Chairpersons: S. Morrison, *HHMI/University of Michigan, Ann Arbor;* R. Weinberg, *Whitehead Institute, Cambridge, Massachusetts*

Panel Discussion/Debate: Stemness Versus Plasticity: What Drives and Maintains Cancer?
Moderators: N. Polyak, S. Muthuswamy
Panelists: S. Morrison, J. Dick, R. Weinberg, A. Ashworth, J. Watson

D. Feron, C. Caroll, K. Brindle, G. Evan

Tumor Immunology
Chairpersons: D. Fearon, *University of Cambridge, United Kingdom;* P. Ohashi, *Ontario Cancer Institute, Toronto, Canada*

Tumor-Stroma Interactions: Inflammation
Chairpersons: M. Bissell, *Lawrence Berkeley National Laboratory, Berkeley, California;* V. Weaver, *University of California, San Francisco*

Cancer Cell Metabolism
Chairpersons: T. Mak, *Ontario Cancer Institute, Toronto;* C.V. Dang, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Molecular Imaging and Targeted Drug Delivery
Chairperson: M.C. Simon, *University of Pennsylvania, Philadelphia*

Panel discussion

Telomeres and Telomerase

May 3–7 307 Participants

ARRANGED BY **Titia de Lange,** The Rockefeller University
Roger Reddel, Children's Medical Research Institute
Dorothy Shippen, Texas A&M University
Virginia Zakian, Princeton University

D. Shippen, R. Reddel, T. de Lange, V. Zakian

This conference consisted of eight sessions of talks and two poster sessions. As in 1999, 2001, 2003, 2005, 2007, and 2009, 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 given 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 many 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; diseases associated with short telomeres; telomerase-independent telomere maintenance; telomeric RNA; and effects of telomere dysfunction on mitochondria.

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 2013.

This meeting was funded in part by the National Institute on Aging, a branch of the National Institutes of Health.

T. Cech, B. Stillman

N. Wesolowska, M. Mefford

PROGRAM

Chairpersons: P. Baumann, *Stowers Institute for Medical Research, Kansas City, Missouri;* C. Greider, *Johns Hopkins University, Baltimore, Maryland*

Telomerase Biochemistry and Regulation
Chairpersons: E. Blackburn, *University of California, San Francisco;* S. Artandi, *Stanford University School of Medicine, California*

Telomeric Proteins
Chairpersons: V. Lundblad, *Salk Institute for Biological Studies, La Jolla, California;* S. Smith, *Skirball Institute, New York University, New York*

Consequences of Telomere Dysfunction
Chairpersons: M. Longhese, *Università di Milano-Bicocca, Italy;* J. Shay, *University of Texas Southwestern Medical Center, Dallas*

CST and the Telomeric Overhang
Chairpersons: T. Cech, *HHMI/University of Colorado, Boulder*; F. Ishikawa, *Kyoto University, Japan*

ALT and Telomere Recombination
Chairpersons: T. Cech, *HHMI/University of Colorado, Boulder*; F. Ishikawa, *Kyoto University, Japan*

Telomeres and Disease
Chairpersons: S. Chang, *Yale University, New Haven, Connecticut;* M. Lei, *University of Michigan, Ann Arbor*

Telomere Proteins and Telomere Structure
Chairpersons: P. Lansdorp, *BC Cancer Research Centre, Vancouver, Canada;* J. Lingner, *École Polytechnique Fédérale de Lausanne, Switzerland*

Telomeric RNA
Chairpersons: D. Wuttke, *University of Colorado, Boulder;* M. Bessler, *Children's Hospital of Philadelphia, Pennsylvania*

Telomeres and Mitochondria
Chairpersons: D. Wuttke, *University of Colorado, Boulder;* M. Bessler, *Children's Hospital of Philadelphia, Pennsylvania*

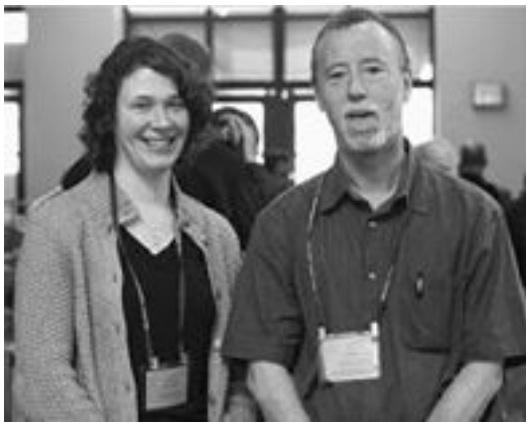
M. Rubtsova

J. Shay, M. Tarsounas

Honey Bee Genomics and Biology

May 8–10 105 Participants

ARRANGED BY **Christina Grozinger,** Penn State University
Uli Mueller, Saarland University
Robert Page, Arizona State University



C. Grozinger, U. Mueller

This meeting was well attended by researchers representing 15 countries. The conference began with keynote presentations by Hugh Robertson, Andrew Feinberg, and Martin Beye. These speakers, respectively, discussed comparative genomic approaches to the honey bee chemosensory system, including comparisons to six newly sequenced ant genomes; the role of DNA methylation in regulating honey bee worker behavioral plasticity; and an elegant molecular and evolutionary analysis of the sex determination pathway in honey bees. The scientific sessions and their chairpersons are listed on the following page. The poster session included 41 additional presentations of these topics as well. The conference was preceded by a National Science Foundation (NSF)-supported workshop on the "Development of Functional Genetic Tools in Honey Bees," which included several presentations and discussions of the utility of RNA interference (RNAi), cell culture, and other approaches to dissecting the genetic underpinnings of behavior, physiology, and development in bees. During the wrap-up session, there was considerable enthusiasm for the conference—several participants felt this was the best conference that they had attended since the 2007 meeting—and plans were developed to expand the conference to include other social insects with sequenced genomes.

This meeting was funded in part by the University of Illinois; the National Science Foundation; and DNAFORM.

G. Thompson, A. Camiletti

A. Mushegian

PROGRAM

Plenary Lectures
H.M. Robertson, *University of Illinois, Urbana-Champaign*
A.P. Feinberg, *Johns Hopkins University School of Medicine*
M. Beye, *Heinrich-Heine University, Dusseldorf, Germany*

Epigenetics and Genomics
Chairpersons: S. Yi, *Georgia Institute of Technology, Atlanta;* G. Hunt, *Purdue University, West Lafayette, Indiana*

Neurobiology and Behavior
Chairpersons: D. Eisenhardt, *Freie Universität Berlin, Germany;* R. Scheiner, *Universität Potsdam, Germany*

Physiology and Development
Chairpersons: G. Amdam, *Arizona State University, Tempe;* G. Bloch, *Hebrew University of Jerusalem, Israel*

Immunity and Host–Parasite Interactions
Chairpersons: J. Evans, *USDA-ARS, Beltsville, Maryland;* E. Genersch, *Länderinstitut für Bienenkunde, Hohen Neuendorf, Germany*

Population Genetics, Evolutionary, and Comparative Genomics
Chairpersons: C. Whitfield, *University of Illinois, Urbana-Champaign*; J. Gadau, *Arizona State University, Tempe*

Plenary Lecture: The Blind Men and the Elephant: Genes, Neurons, and the Brain
G.E. Robinson, *University of Illinois, Urbana-Champaign*

K. Thle, C. Narraway

M. Beye, O. Rueppell

The Biology of Genomes

May 10–14 550 Participants

ARRANGED BY **Susan Celniker,** Lawrence Berkeley National Laboratory
Andrew Clark, Cornell University
Joel Hirschhorn, The Broad Institute of Harvard/Massachusetts Institute of Technology
Chris Ponting, UK Medical Research Council, University of Oxford

This annual meeting marked the 24th annual gathering of genome scientists at Cold Spring Harbor Laboratory. Just over 550 people from around the world attended the meeting, with more than 350 abstracts presented describing a broad array of topics relating to the production, analysis, and interpretation of genomes from diverse organisms. The meeting built on the remarkable progress in the sequencing, functional annotation, and analysis of genomes from many human individuals, "model organisms," other animals, plants, microbiomes, and prokaryotes. Many investigators reported on their application of the most recent generation of DNA sequencing technologies to determine sequence variants within populations that convey differences in molecular function and organismal phenotype and in susceptibility to disease, including cancer and complex disease. The new data are also providing important insights into functional genomic elements and population structure for humans and other species. Several investigators reported important differences in cell-type-specific transcriptome maps and epigenomic profiles. Newly sequenced primate, lizard, and rodent genomes were also announced.

C. Ponting, J. Hirschhorn, S. Celniker, A. Clark

There were numerous reports on progress in using the sequencing technologies, with mounting expectation for further advances as these continue to be applied widely. The Keynote Addresses were delivered by Andrew Feinberg and Susan Lindquist. The ELSI (Ethical, Legal, and Social Implications) panel was moderated by Pilar Ossorio, and the topical area of discussion was the "Ethical,

B. Korberg, B. Dorshorst

Legal, and Social Issues in Genetics and Genomics Research with Indigenous People."

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health; Agilent Technologies; BioNanomatrix; Complete Genomics; DNAFORM; EdgeBio; Epigentek; Gene Link; Illumina; Innopsys; Molecular Devices; NimbleGen; and 454 Life Sciences, a Roche company.

PROGRAM

High-Throughput Genomics and Genetics
Chairpersons: K. Gunsalus, *New York University;* J. Lieb, *University of North Carolina, Chapel Hill*

Genetics of Complex Traits
Chairpersons: C.A. Kong, *deCODE Genetics, Reykjavik, Iceland;* P. Visscher, *Queensland Institute of Medical Research, Brisbane, Australia*

Functional and Cancer Genomics
Chairpersons: J. Stamatoyannopoulos, *University of Washington, Seattle;* G. Getz, *Broad Institute of MIT and Harvard, Cambridge, Massachusetts*

Computational Genomics
Chairpersons: R. Bonneau, *New York University;* K. Makova, *Pennsylvania State University, University Park*

ELSI Panel and Discussion: Ethical, Legal, and Social Issues in Genetics and Genomics Research with Indigenous People
Moderator: P.N. Ossorio, *University of Wisconsin*
Panelists: H. Soodyall, *University of the Witwatersrand*
M.L. Tano, *International Institute for Indigenous Research Management*
L.T. Arbour, *University of British Columbia*
R.J. Whitener, *University of Washington*

Keynote Address
A. Feinberg, *Johns Hopkins University School of Medicine*

Evolutionary Genomics
Chairpersons: A. McLysaght, *University of Dublin-Trinity College, Ireland;* D. Stern, *HHMI/Princeton University, New Jersey*

Population Genomic Variation
Chairpersons: D. Reich, *Harvard Medical School, Boston, Massachusetts;* G. Coop, *University of California, Davis*

Keynote Address
S. Lindquist, *Whitehead Institute for Biomedical Research*

Genetics and Genomics of Nonhuman Species
Chairpersons: B. Andrews, *University of Toronto, Canada;* Y. Gilad, *University of Chicago, Illinois*

D. Schwartz, R. Meyers

E. Nickerson, D. Ware

F. Casals, D. Comas

The Ubiquitin Family

May 17–20 313 Participants

ARRANGED BY **J. Wade Harper,** Harvard Medical School
Ronald Hay, University of Dundee
Ron Kopito, Stanford University
Brenda Schulman, Memphis, Tennessee

This fifth meeting reprises the successful inauguration of this series in 2003. It focused on ubiquitin, 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.

R. Hay, J.W. Harper, R. Kopito, B. Schulman

The meeting attracted more than 310 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, which 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 fungi, plants, and mammals, were used to probe functions

C. Dahlberg

R. Hjerpe, D. Xirodimas, Y. Thomas

R. Milkereit

of ubiquitin and ubiquitin-like proteins in diverse physiological processes ranging from control of stem cell division, DNA repair, and pathways associated with diseases such as neurological disorders and cancers. Other highlights of the meeting this year concerned progress on the use of mass spectrometry to both define the ubiquitin and SUMO-modified proteomes and understand how these vary in cellular responses to different signals. Significant advances were also reported in terms of the structures and mechanisms of enzymes that function in ubiquitin conjugation and deconjugation, with the emerging understanding of how enzyme modification, such as by acetylation and phosphorylation, influences conjugation and deconjugation activities. Fascinating new structural mechanisms were reported that explain how different types of ubiquitin chains, and ubiquitin-like protein modifications—and their dynamic properties—impart new functionalities to target proteins. Finally, mechanism and physiology converged in several presentations describing exciting new ways in which small molecules can target the ubiquitin proteasome at virtually every level of the pathway. In summary, this meeting has continued to be a unifying forum that helps us to understand the regulatory mechanisms by which ubiquitin and ubiquitin-like proteins function, their ever-growing roles in cellular physiology and disease, and the development of therapeutics targeting the ubiquitin family.

I.F. Melandri, J. Yin, K. Kluge

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; Boston Biochem; Millenium Pharmaceuticals; Novartis; and Ubiquigent.

PROGRAM

Conjugation, Removal, and Recognition of Ubiquitin and Ubiquitin-Like Proteins I
Chairpersons: C. Lima, *Memorial Sloan-Kettering Institute, New York;* R. Klevit, *University of Washington, Seattle*

Keynote Address
R. Deshaies, *California Institute of Technology*

Conjugation, Removal, and Recognition of Ubiquitin and Ubiquitin-Like Proteins II
Chairpersons: N. Zheng, *University of Washington, Seattle;* D. Morgan, *University of California, San Francisco*

Substrate Targeting and Degradation
Chairpersons: D. Finley, *Harvard Medical School, Boston, Massachusetts;* M. Hochstrasser, *Yale University, New Haven, Connecticut*

UBLs in Signaling I: Receptors, Plasma Membrane/ Endosomal Trafficking
Chairpersons: S. Urbe, *University of Liverpool, United Kingdom;* A. Smogorzewska, *The Rockefeller University, New York*

Quality Control
Chairpersons: R. Hampton, *University of California, San Diego;* I. Dikic, *Goethe University Medical School, Frankfurt, Germany*

Nuclear Functions of UBLs
Chairpersons: C. Wolberger, *HHMI/Johns Hopkins University School of Medicine, Baltimore, Maryland;* F. Melchior, *ZMBH, Heidelberg, Germany*

Regulation of Intracellular Pathways
Chairpersons: M. Tyers, *Mount Sinai Hospital, Toronto, Canada;* M. Rape, *University of California, Berkeley*

Keynote Address
V. Dixit, *Genentech, Inc.*

UBLs in Signaling II: Intracellular Communication and Regulation of Organismal Biology
Chairpersons: A. Bonni, *Harvard Medical School, Boston, Massachusetts,* M.G. Mascucci, *Karolinska Institute, Stockholm, Sweden*

Retroviruses

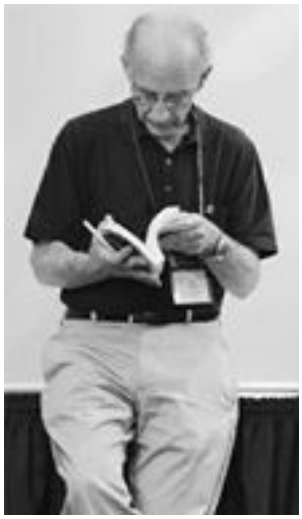
May 23–28 428 Participants

ARRANGED BY **Henry Levin,** National Institute of Health
Eric Poeschla, Mayo Clinic College of Medicine

This 36th annual meeting had all of the characteristics that have long made it the most important one for retrovirologists: the presence of the field's top scientists along with many students and young investigators, presentations of cutting-edge unpublished data, exuberant poster sessions, and the announcement of at least one landmark discovery.

The participants, representing 20 countries and six continents, gave 125 oral and 187 poster presentations. Following tradition, the 10 oral and three poster sessions focused on unpublished, basic research on retrovirus replication and on cellular factors exploited by viruses or deployed by hosts in defense. Lentiviruses, most prominently HIV-1, took center stage, but all seven genera were represented. Innate (intrinsic) immunity mechanisms, most notably restriction factors, were a strong theme, and the revelation of a new one, SAMHD1, garnered particular attention.

N. Laguette and K. Hrecka presented the SAMHD1 data, thereby identifying the long-sought target of Vpx. These findings updated the theme featured on the meeting book cover: Sensors and Sensing in Innate Antiviral Immunity. Vpx, present in some simian lentiviruses and HIV-2, enables myeloid cell infection. Fittingly, the Andy Kaplan Prize was awarded to N. Manel for his 2010 work showing that Vpx allows infection of human dendritic cells (DCs), whereupon a DC sensor that triggers a type I interferon response is activated. The current meeting papers showed that Vpx does this by loading the restriction factor SAMHD1 onto the CRL4/DCAF1 E3 ubiquitin ligase, triggering its degradation.


P. Jolicoeur

A second focus of attention was XMRV, a γ retrovirus recently implicated in a human disease (prostate cancer) and syndrome (chronic fatigue). Here, though, a series of talks presented compelling evidence that XMRV is, in fact, not a human pathogen. Rather, it arose quite recently, in the laboratory, by recombination between two endogenous murine precursors when human prostate cancer cell xenografts were passaged in mice (Cingöz, Hué, Paprotka). It is not detected in well-defined American and German cohorts subjected to properly controlled, sensitive assays (Hohn, Ikeda). Antibodies raised by injection of human-cell-produced virus into rabbits, previously thought to be XMRV-specific and hence of use in probing human tissue for this virus, were shown to instead cross-react with unknown human proteins (Ikeda). At the close of the session, moderator G. Towers ably summarized the field and explained how the totality of present evidence does not support involvement in human illness.

The poster sessions were intensively attended and a prize for the highest impact poster, the Dan Wolf Memorial prize, followed. It was awarded to T. Schaller for work on a role for RanBP2/Nup358 in nuclear import of HIV-1.

The invited Keynote Speakers were Robert F. Siliciano, who spoke on "Quantitative analysis of the efficacy of antiretroviral drugs and HIV eradication strategies," which illuminated fundamental and sometimes counterintuitive kinetic aspects of drug action and implications for proviral eradication, and Steven J. Elledge, who spoke on "Genetic approaches to virus function," which considered the promise of recent genome-wide screens for understanding RNA viruses and extension of such screens to identify antiviral drugs.

Some further highlights included multiple presentations on Tetherin, including a Tetherin knockout mouse and evidence for enhancement of cell-to-cell spread of FIV by Tetherin; demonstration that cellular CBFβ is an HIV Vif cofactor required for APOBEC3G degradation; characterization

of TREX1 exonuclease targeting cytosolic HIV-1 DNA; several genome and proteome screens that examined host factors including those involved in interferon response gene repertoires; reports on roles in uncoating and PIC trafficking for various nucleoporins, TNP03, CPSF6, and microtubule-associated proteins; evidence for cold inactivation as a significant HIV-1 Env property; studies of SUMO-interacting motifs in TRIM5α and sumoylation of integrase; characterization of the assembly of a hexagonal TRIM5α lattice on HIV-1 capsid; determination of a role for reverse transcription in facilitating uncoating; studies of assembly in macrophages and other cells; evidence that HIV-1 can replicate in ferret cells if receptors are provided; and studies of base-excision repair proteins in integration, regulation of foamy virus Gag nuclear localization, and protease activation by viral RNA. Many other noteworthy advances are not listed here due to space constraints.

This meeting was funded in part by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health.

PROGRAM

Env in Entry, Assembly, and Cell-to-Cell Transmission
Chairpersons: M. Johnson, *University of Missouri, Columbia;* D. McDonald, *Case Western Reserve University, Cleveland, Ohio*

To the Nucleus I: Uncoating, Trim, Vpx/Myeloid
Chairpersons: Z. Ambrose, *University of Pittsburgh, Pennsylvania;* N. Yan, *University of Texas Southwestern Medical Center, Dallas*

To the Nucleus II: Nuclear Import, Host Factors
Chairpersons: E. Bacharach, *Tel Aviv University, Israel;* C. Aiken, *Vanderbilt University, Nashville, Tennessee*

Keynote Address: Quantitative Analysis of the Efficacy of Antiretroviral Drugs and HIV Eradication Strategies
R. Siliciano, *HHMI/Johns Hopkins University School of Medicine*

APOBEC, Genome/Proteome Screens
Chairpersons: R. Harris, *University of Minnesota, Minneapolis;* A. Brass, *Ragon Institute of MGH/MIT/Harvard University, Charlestown, Massachusetts*

Integration
Chairpersons: M. Kvaratskhelia, *Ohio State University, Columbus;* G. Maertens, *Imperial College London, United Kingdom*

Reverse Transcriptase, RNA, and Encapsidation
Chairpersons: L. Parent, *Pennsylvania State University College of Medicine, Hershey;* B. Wöhrl, *University of Bayreuth, Germany*

Assembly I
Chairpersons: D. Lindemann, *Technische Universität Dresden, Germany;* E. Freed, *NCI-Frederick, Maryland*

Keynote Address: Genetics Approaches to Virus Function
S. Elledge, *Harvard Medical School*

Assembly II: Tetherin
Chairpersons: D. Evans, *Harvard Medical School, Boston, Massachusetts;* A. Ono, *University of Michigan Medical School, Ann Arbor*

XMRV, Transcription
Chairpersons: G. Towers, *University College London, United Kingdom;* L. Weinberger, *University of California, San Diego*

Pathogenesis and More
Chairpersons: S. Barr, *University of Western Ontario, London, Canada;* K. Strebel, *NIAID/National Institutes of Health, Bethesda*

K. Strebel, H. Hofmann

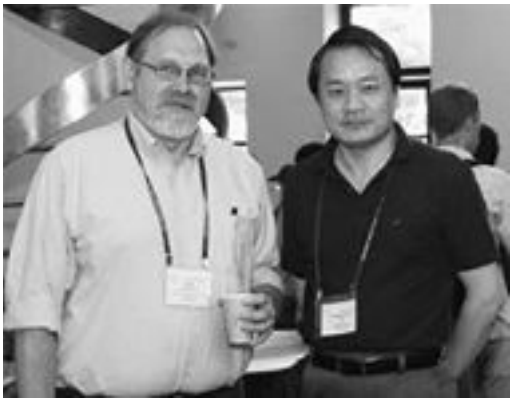
V. KewalRamani, S. Hughes

Single-Cell Analysis

July 22–24 110 Participants

ARRANGED BY **James Eberwine,** University of Pennsylvania Medical School
Xiaoliang Sunney Xie, Harvard University

The goal of this second workshop was to bring together investigators who work with single cells using different experimental paradigms to discuss the progress that is being made. Nearly 112 scientists convened for 31 talks and 22 posters. There was a panoply of cell types 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, single-cell genomic DNA sequencing, proteomics, and metabolomics, all at single-cell resolution. A common theme from many of the talks and posters was individual cell-to-cell variability in mRNA abundances, DNA sequence, and protein expression. An important component of the meeting was discussion of various imaging modalities, including single-molecule-resolution optics using PALM and STORM methodologies. 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 constitute a multicellular organism, the discipline of computational biology as an organizing and analytical 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 states, better production of biofuels, and insight into evolutionary mechanics that is unachievable using pooled cells.



J. Eberwine, X.S. Xie

This meeting was funded in part by Applied Biosystems, Fluidigm, Illumina, Molecular Devices, Olympus, the University of Pennsylvania, and Carl Zeiss, Inc.

PROGRAM

Single-Cell Analyses: Genomes
Chairperson: J. Eberwine, *University of Pennsylvania Medical School, Philadelphia*

Keynote Address
X. Zhuang, *Harvard University*

Single-Cell Analyses: Transcriptomics
Chairperson: X.S. Xie, *Harvard University, Cambridge, Massachusetts*

Single-Cell Analyses: Proteomics
Chairperson: S. Fraser, *California Institute of Technology, Pasadena*

Single-Cell Analyses: Imaging
Chairperson: J. Sweedler, *University of Illinois, Urbana-Champaign*

Single-Cell Analyses: Modeling
Chairperson: L. Cai, *California Institute of Technology, Pasadena*

Yeast Cell Biology

August 16–20 219 Participants

ARRANGED BY **Kerry Bloom,** University of North Carolina, Chapel Hill
Martha Cyert, Stanford University
Lois Weisman, University of Michigan

This was the 14th biannual international meeting devoted to diverse aspects of cell biology in yeasts. Studies of these simple eukaryotes continue to generate key insights into fundamental, conserved aspects of cell function. The meeting highlighted the combined use of mathematical modeling and quantitative imaging of fluorescently labeled proteins in live cells to understand complex biological processes such as cytokinesis and endocytosis. T. Pollard's and R. Li's labs (using *S. pombe* and *S. cerevisiae*, respectively) are constructing detailed mechanistic models that explain the dynamic behavior of actin and myosin during the assembly and contraction of the cytokinetic ring. Stunning progress in this area is a testament to the enduring power of yeast as an experimental organism for cell biology. M. Kaksonen's lab used fluorescence cross-correlation spectroscopy to measure precise protein concentrations and interactions in vivo for key components of endocytosis. J. Skotheim, a mathematician recently recruited to cell biology, used single-cell analyses to quantitatively describe mating-factor-induced cell cycle arrest. Studies such as these elucidate macroscopic cell behaviors at a mechanistic level and are revealing that common regulatory principles underlie diverse biological responses. Quantitative live-cell imaging at a systems level was discussed by B. Andrews, who determined a "subcellular flux network" for ~3000 green fluorescent protein (GFP)-labeled yeast proteins that describes how each protein changes in abundance or localization under several different conditions and offers biologists an integrated, dynamic view of protein function within the cell.

M. Cyert, K. Bloom, L. Weisman

J. Johnson, M. Lord

B. Andre, P. Lungdahl

Regulation of protein function by posttranslational modifications was a major topic at the conference. The Gasser lab showed that protein sumoylation causes tethering of telomeres to the nuclear envelope and that short telomeres dissociate from the nuclear envelope when they are elongating. Thus, telomere anchoring, mediated by sumoylation, may regulate telomerase activity, and similar observations in *C. elegans* suggest that this mechanism is conserved. The growing role of acetylation in modifying protein function was highlighted (Baetz and Strich labs) and insights into the recognition of protein targets by kinases and phosphatases were presented (Pryciak, Weiss, Turk, Stuart, and Cyert labs).

Other topics included chromosome instability and its consequences in yeast and cancer cells. Inhibition of Hsp90 increases chromosome loss (R. Li's lab), and the resulting aneuploidy facilitates the rapid acquisition of novel traits, including resistance to several drugs. Aneuploid yeast strains also have high levels of genomic instability due to increased chromosome loss and recombination and impaired DNA-damage repair (Amon lab). Thus, by analogy, changes in the karyotype of human cells may profoundly influence the evolution of cancer cells. Mutations in the yeast mRNA processing machinery also result in chromosome instability and DNA damage that may be caused by persistence of RNA:DNA hybrids (Hieter lab). These studies suggest new insights into human leukemias, in which similar mutations occur.

This intensely enjoyable conference united scientists from disparate areas of cell biology who share common organism-specific approaches. As always, this cross-fertilization revealed surprising new connections between well-studied processes and will stimulate the next generation of advances in cell biology.

This meeting was funded in part by the National Institute of General Medical Sciences, a branch of the National Institutes of Health; the National Science Foundation; CellASIC; and Molecular Devices.

PROGRAM

Membranes and Lipids
Chairpersons: M. Hochstrasser, *Yale University, New Haven, Connecticut;* A. Spang, *Max-Planck Society, Tübingen, Germany*

Chromosomes and Mitosis
Chairpersons: P. Hieter, *University of British Columbia, Vancouver;* A. Amon, *Massachusetts Institute of Technology, Cambridge*

Cell Cycle and Growth
Chairpersons: D. Botstein, *Princeton University, New Jersey;* D. Pellman, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Membrane Trafficking
Chairpersons: Y. Ohsumi, *Okazaki National Research Institutes, Japan;* M. Munson, *University of Massachusetts Medical School, Worcester*

Informal Discussion on Funding for Yeast Research
Led by S. Sazar, *National Science Foundation*

Nucleus and Cytoplasmic Organelles
Chairpersons: S. Wente, *Vanderbilt University Medical Center, Nashville, Tennessee;* K. Cunningham, *Johns Hopkins University, Baltimore, Maryland*

Signaling Networks
Chairpersons: J. Weissman, *HHMI/University of California, San Francisco;* R. Li, *Stowers Institute for Medical Research, Kansas City, Missouri*

Genomes and Gene Expression
Chairpersons: B. Andrews, *University of Toronto, Canada;* J. Haber, *Brandeis University, Waltham, Massachusetts*

Polarity and Cytokinesis
Chairpersons: T. Pollard, *Yale University, New Haven, Connecticut;* F. Verde, *University of Miami, Florida*

Eukaroytic mRNA Processing

August 23–27 371 Participants

ARRANGED BY **Tom Blumenthal,** University of Colorado
Kristen Lynch, University of Pennsylvania
Karla Neugebauer, MPI-CBG

This eighth 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).

K. Lynch, K. Neugebauer, T. Blumenthal

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 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 RNPs (hnRNPs) 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

Global Analysis: RNA Processing/Global Analysis: RNPomics
Chairpersons: L. Chasin, *Columbia University, New York;* J. Sanford, *University of California, Santa Cruz*

Spliceosome Assembly/Spliceosome Function
Chairpersons: J. Staley, *University of Chicago, Illinois;* D. Brow, *University of Wisconsin, Madison*

3′-End Processing/Translation
Chairpersons: J. Wilusz, *Colorado State University, Fort Collins;* G. Carmichael, *University of Connecticut Health Center, Farmington*

RNAi and miRNAs/ncRNAs
Chairpersons: A. Pasquinelli, *University of California, San Diego;* N. Kim, *Seoul National University, Korea*

Molecular Interactions during RNA Processing/Regulation of RNA Processing
Chairpersons: C. Kielkopf, *University of Rochester, New York;* K. Hertel, *University of California, Irvine*

Cotranscriptional Pre-mRNA Processing I/ Cotranscriptional Pre-mRNA Processing II
Chairpersons: D. Bentley, *University of Colorado School of Medicine, Aurora;* J. Beggs, *University of Edinburgh, United Kingdom*

Nuclear RNA Degradation/Cytoplasmic RNA Surveillance and Localization
Chairpersons: D. Tollervey, *University of Edinburgh, United Kingdom;* A. Ephrussi, *EMBL, Heidelberg, Germany*

Splicing Regulatory Mechanisms/Biological Function of RNA Processing
Chairpersons: C. Smith, *University of Cambridge, United Kingdom;* T. Cooper, *Baylor College of Medicine, Houston, Texas*


E. Lund, J. Caceres


M. Umemura


K. Hartmuth

Mechanisms of Eukaryotic Transcription

Aug. 30–Sept. 3 386 Participants

ARRANGED BY **Stephen Buratowski,** Harvard Medical School
Steven Hahn, Fred Hutchinson Cancer Research Center
Katherine Jones, The Salk Institute for Biological Studies

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 12th meeting appropriately covered many aspects of the field and brought together a diverse group of scientists. It consisted of eight plenary sessions, three poster sessions, and two breakout sessions.

S. Buratowski, S. Hahn, K. Jones

The meeting began with a new session—"Transcription control of cell fate"—that described mechanisms controlling stem cell identity and key developmental decisions. Described in other sessions were studies at the forefront of understanding the mechanisms used for transcription initiation by all three eukaryotic and the archaeal RNA polymerases; mechanisms regulating the targeting and activity of factors, in addition to systems biology approaches for understanding how gene networks are coordinated to direct complex biological processes; and new studies on the many fascinating ways in which modulation of chromatin can contribute to gene control. Another session showed the importance of regulating polymerase elongation and the many mechanisms that contribute to this process, and yet another presented the latest findings on conserved coactivator complexes including their structure, function, and interaction with activation domains. The elongation theme continued and was primarily focused on cotranscriptional events, such as nucleosome modification and remodeling, that are linked to elongation. A final session described transcription defects directly causing human disease, drugs targeting key transcription components, and the role of noncoding RNAs that regulate transcription in unexpected ways.

A. Bataile, C. Jeronimo, F. Robert

E. Rosonina

Interspersed with these oral presentations were the three poster sessions, where a wide variety of the latest transcriptional research was presented. New for the meeting was the inclusion of two breakout sessions that were specifically requested by meeting participants as a way to supplement the oral and poster sessions and to give an opportunity for junior scientists to present their work. From discussions with attendees of these sessions, they were a great success. It is hoped that they will be continued at the next meeting on topics yet to be determined. This meeting was very well received by the participants and will occur again in 2013.

The meeting was funded in part by a grant from the National Science Foundation.

PROGRAM

Transcription Control of Cell Fate
Chairperson: B. Cairns, *University of Utah, Salt Lake City*

Initiation and Promoter Escape
Chairperson: J. Goodrich, *University of Colorado, Boulder*

Regulation of Transcription Factor Activity and Specificity
Chairperson: M. Levine, *University of California, Berkeley*

Chromatin
Chairperson: C. Wolberger, *HHMI/Johns Hopkins University School of Medicine, Baltimore, Maryland*

Elongation Mechanisms
Chairperson: K. Luger, *HHMI/Colorado State University, Fort Collins*

Coactivators
Chairperson: M. Timmers, *UMC Utrecht, the Netherlands*

Elongation and Coupled Events
Chairperson: I. Grummt, *German Cancer Research Center, Heidelberg*

Metabolism and Disease/Noncoding RNAs
Chairperson: S. Mango, *Harvard University, Cambridge, Massachusetts*

M. Levine, K. Struhl

D. Singer, C.M. Chiang

A. Cheung, F. Martinez

Eukaryotic DNA Replication and Genome Maintenance

September 6–10 335 Participants

ARRANGED BY **Stephen Bell,** HHMI/Massachusetts Institute of Technology
Joachim Li, University of California, San Francisco
Johannes Walter, Harvard Medical School

This 12th biannual meeting reflected the remarkable progress that has been made in the past 2 years and served an important role in bringing together researchers studying eukaryotic DNA replication, repair, and their cell cycle control. As in the past, the meeting brought together an international cadre of researchers who presented exciting new advances upholding the tradition that this is the most important meeting in the DNA replication field. A total of 331 investigators participated in the 10 scientific sessions, with 262 platform and poster presentations. The eight platform sessions were marked by spirited and enthusiastic exchanges of new results. The poster sessions were of very high quality and were overcrowded with interested attendees delving into the presented findings.

S. Bell, J. Walter, J. Li

Sessions at the meeting included novel approaches to understanding DNA replication; origin selection and helicase loading; responses to replication stress and damaged DNA; origin activation and replication timing; chromatin, cancer, and development; replisome function; and cell cycle control and connections. The presentations illustrated the growing power of biochemical reconstitution and single-molecule analysis in dissecting the mechanisms of cellular DNA replication initiation and repair. Genome-wide and replication-fork-specific assays showed new promise for understanding the mysterious determinants for origin selection in metazoan cells. The important influences of replication protein abundance, chromosome folding, chromatin structure in the determination of origin activation, and replication fork function were reported. In addition, the presentations illustrated our increasing understanding of the close coordination between the DNA repair and cell cycle checkpoints 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.

E. Leo

A.M. Mass, R. Muller, K. Gilljam, J. Kusic

D. Sherratt, J. Blow

A. Schwacha, G. Kapler

PROGRAM

New Aproaches and Views of Replication
Chairpersons: D. MacAlpine, *Duke University Medical Center, Durham, North Carolina;* D. Sherratt, *University of Oxford, United Kingdom*

Origin Selection and Pre-RC Assembly
Chairpersons: J. Blow, *University of Dundee, United Kingdom;* M. Debatisse, *Institut Curie-CNRS-UPMC, Paris, France*

Response to Replication Stress and Damage
Chairpersons: K. Cimprich, *Stanford University, California;* A. Carr, *University of Sussex, Brighton, United Kingdom*

Origin Activation and Timing
Chairpersons: J. Berger, *University of California, Berkeley;* J. Diffley, *Cancer Research UK London Research Institute, South Mimms*

Chromatin, Cancer, and Development
Chairpersons: N. Francis, *Harvard University, Cambridge, Massachusetts;* M. Jasin, *Memorial Sloan-Kettering Cancer Center, New York*

Replisome Activities and Functions
Chairpersons: M. Botchan, *University of California, Berkeley;* E. Fanning, *Vanderbilt University, Nashville, Tennessee*

Cell Cycle Control and Connections
Chairpersons: J. Cook, *University of North Carolina, Chapel Hill;* B. Stillman, *Cold Spring Harbor Laboratory*

Postreplicative Repair and Lesion Bypass
Chairpersons: H. Ulrich, *Cancer Research UK London Research Institute, South Mimms;* G. Walker, *Massachusetts Institute of Technology, Cambridge*

R. Müller

Microbial Pathogenesis and Host Response

September 13–17 277 Participants

ARRANGED BY **Andrew Camilli,** Tufts University School of Medicine
Brendan Cormack, Johns Hopkins University School of Medicine
Lalita Ramakrishnan, University of Washington

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, 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 the microbial–host interaction surface, regulation of virulence gene expression, microbial metabolism and physiology, innate immune signaling, immune evasion strategies, evolution of virulence, evolution of the host immune response, 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. Ralph Isberg, an internationally recognized leader in microbial pathogenesis, presented the Keynote Address on how adaptation of *Legionella pneumophila* to survival in diverse amoebae has shaped the evolution of the pathogen and trained it to survive in alveolar macrophages in humans.

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, which followed all oral presentations, was also evident

A. Huerta-Saquero, S. Hernandez, J. Santander, I. Ferlenghi

O. Shannon

throughout the poster 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, a branch of the National Institutes of Health.

PROGRAM

Microbial Structures
Chairperson: S. Hultgren, *Washington University School of Medicine, St. Louis, Missouri*

Virulence Regulation
Chairperson: W. Bishai, *Johns Hopkins University School of Medicine/K-RITH, Baltimore, Maryland*

Signaling: Host and Microbe
Chairperson: T. Koehler, *University of Texas, Houston Medical School*

Intracellular Lifestyles
Chairperson: K. Kuchler, *Medical University Vienna, Austria*

The Cell Envelope and Beyond
Chairperson: T. Silhavy, *Princeton University, New Jersey*

Immunity
Chairperson: D. Littman, *HHMI/New York University School of Medicine*

Keynote Address: *Legionella,* a Pathogen and a Generalist
R.R. Isberg, *Tufts University School of Medicine*

Evolution: Host and Pathogen
Chairperson: J. Ernst, *New York University School of Medicine*

T. Costa, P. Paracuellos

A. Camilli, P. Sundstrom, J. Slauch

Stem Cell Biology

September 20–24 190 Participants

ARRANGED BY **Konrad Hochedlinger,** Massachusetts General Hospital
Brigid Hogan, Duke University Medical Center
Allan Spradling, HHMI/Carnegie Institution

This second meeting followed the same basic format that distinguished it 2 years ago. Structurally, this involved relatively short talks of 20–25 minutes in length, followed by at least 5 minutes of discussion. Often, the discussion was very lively and lasted 10 minutes or more. Nonetheless, the sessions stayed largely on time because the number of speakers was limited to about six. The meeting differed from many others on this topic in a number of ways. The focus was very much on the molecular and developmental biology of tissue stem cells in vivo and the molecular controls of cell differentiation and dedifferentiation. In contrast, there were few descriptive presentations that had not yet yielded mechanistic insights. In addition, insights from a wide range of relevant systems were presented, including three talks on plants and one on lamprey development. The best-characterized tissue stem cells in a range of organisms were discussed from the perspectives of their molecular regulation and cell biological mechanisms. Key subject areas of the meeting were the stem cell niche, asymmetric cell division, stem cell differentiation, reversion to the stem cell state, tissue repair, regeneration and reprogramming, epigenetics of stem cells, and pluripotency. Talks contained a substantial fraction of unpublished material, and several findings were presented for the first time. The novelty of the work was reflected by the increased attendance by journal representatives. The Keynote Address by Elaine Fuchs was a major highlight and was discussed actively for more than 25 minutes, before participants were asked to clear the hall. Informal feedback suggested that the participants were extremely positive about the value of the experience. Speaker comments included "I have been

A. Spradling, B. Hogan, K. Hochedlinger

Poster session

learning a lot," "this is the best meeting I have been to all year," and "I'm so glad that you included talks from such a diversity of systems." While echoing these sentiments, students and postdocs were also extremely pleased by the accessibility of speakers and the depth with which they were able to discuss relevant issues both after the talks and during the two poster sessions. "I learned more from the discussion than from the talks." "I just spent 30 minutes discussing my project with Elaine Fuchs!"

This meeting was funded in part by Fluidigm and Molecular Devices.

PROGRAM

Stem Cell Niches I
Chairperson: A. Spradling, *HHMI/Carnegie Institution, Baltimore, Maryland*

Asymmetric Cell Division
Chairperson: A. Brand, *University of Cambridge, United Kingdom*

Stem Cell Niches II
Chairperson: B. Ohlstein, *Columbia University Medical Center, New York*

Transcriptional and Epigenetic Control of Stem Cell Differentiation and Reversion
Chairperson: K. Hochedlinger, *Harvard Medical School, Boston, Massachusetts*

Tissue Repair, Regeneration, and Reprogramming
Chairperson: B. Hogan, *Duke University Medical Center, Durham, North Carolina*

Metabolism and Noncoding RNAs in Stem Cell Regulation
Chairperson: E. Fuchs, *HHMI/University of California, San Francisco*

Keynote Address: Skin Stem Cells in Normal Homeostasis, Wound Repair, and Cancer
E. Fuchs, *HHMI/The Rockefeller University, New York*

Pluripotency and Epigenetics
Chairperson: Y. Yamashita, *University of Michigan, Ann Arbor*

Y. Fung, T. Yamaguchi

Q. Lu, J. Wen, M. Qiu

Y. Yamashita

Personal Genomes

Sept. 30–Oct. 2 180 Participants

ARRANGED BY **Paul Flicek,** European Bioinformatics Institute
James Lupski, Baylor College of Medicine
Elaine Mardis, Washington University School of Medicine

This fourth meeting focused on clinical genome sequencing, the genetic basis of phenotypes, and return of results in the context of continued advances in DNA sequencing technology and its application to clinical medicine. The meeting featured a combination of talks and posters and included three specifically chosen Keynote talks and an Ethics Panel discussion that directly addressed the issue of return of results from personal genome studies and incidental findings. The critical success and timeliness of the meeting are indicative of the fact that the field is changing and the participation of an increased number of attendees from clinical disciplines is apparent. To address these changes and the new-found audience, the collective decision was taken to attempt to merge this meeting with the Pharmogenomics and Personalized Medicine meeting that took place during the same weekend in Hinxton, United Kingdom, and hold the combined meeting under the title "Personal Genomes and Medical Genomics."

This meeting was funded in part by BioNanoMatrix, Complete Genomics, Illumina, and Oxford Nanopore Technologies.

PROGRAM

Personal Cancer Genomics
Chairpersons: E. Mardis, *Washington University School of Medicine, St. Louis, Missouri;* J. Ivanovich, *Washington University, St. Louis, Missouri*

Keynote Address: Cancer Whole-Genome Analysis: Discovery and Clinical Application
E. Mardis, *Washington University School of Medicine, St. Louis, Missouri*

Medically Actionable Genomics
Chairpersons: A. Butte, *Stanford University School of Medicine, California;* D. Dimmock, *Medical College of Wisconsin, Milwaukee*

Personal Genomes
Chairpersons: K. Burns, *Johns Hopkins University School of Medicine, Baltimore, Maryland;* M. Kayser, *Erasmus MC University Medical Center, Rotterdam, The Netherlands*


F. De la Vega, T. Smith


T. Hambuch, G. Lyon


R. Gibbs, J. O'Daniel

Keynote Address
D. Valle, *Johns Hopkins University School of Medicine*

Ethics Panel
Moderator: J. Lupski, *Baylor College of Medicine, Houston, Texas*
Panelists: E. Clayton, *Vanderbilt University;* L. Dressler, *University of North Carolina, Chapel Hill;* B. Eliasen, *Ministry of Health, Faroe Islands;* H. Tabor, *University of Washington, Seattle*

Panel Discussion: Return of Results

Rare Diseases?
Chairpersons: S. Kingsmore, *Children's Mercy Hospital, Kansas City, Missouri;* P. Flicek, *European Bioinformatics Institute, Hinxton, United Kingdom*

Keynote Address: Signs of Change: Personal Genomes as a Commodity
R.A. Gibbs, *Baylor College of Medicine Human Genome Sequencing Center*

Clinical Implementation of Personal Genomics
Chairpersons: M. Boguski, *Harvard Medical School, Boston, Massachusetts;* H. Valantine, *Stanford University, California*

D. Mittleman, M. Ronaghi

M. Skipper, D. Bentley

Neurobiology of *Drosophila*

October 3–7 481 Participants

ARRANGED BY **Nancy Bonini,** HHMI/University of Pennsylvania
Claude Desplan, New York University

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 and the meeting organizers 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. Focal points in the meeting were the Keynote Address by Rüdiger Wehner and the Benzer Lecture by Yuh-Nung Jan. Rüdiger Wehner, a classic figure in insect behavior, described on the first evening his major work on navigation by desert ants and the amazing ability of this insect to find its way without any landmark, simply using the sun as a compass and counting steps for distance. Yuh-Nung Jan, a prominent leader in the field of *Drosophila* neurobiology, gave an historical perspective on *Drosophila* behavior and his many contributions, starting with his work with Seymour Benzer. 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, of the fly—and the design of new technology. A significant shift was clear, where many of the talks con-

A. Pisso, S. Birman

K. O'Connor, J.T. Littleton

P. Haynes, W. Huetteroth

cerned circuits and behavior, rather than development and studies of sensory systems. Cory Root, from Jing W. Wang's lab at the University of California, San Diego, presented the Elkins Plenary Lecture. He described his Ph.D. work on olfactory gain control. 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 toward circuits and behavior demonstrate the vitality of this area. Discussions led to cross-fostering of ideas, which was valuable to everyone in the field.

This 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, and by the National Science Foundation.

PROGRAM

Sensory Systems
Chairperson: T. Clandinin, *Stanford University, California*

Keynote Address: **Beyond *Drosophila:* Maps in Insect Minds?**
R. Wehner, *University of Zürich, Switzerland*

Behavior, Brain Function, and Its Evolution
Chairperson: K. Scott, *University of California, Berkeley*

Neural Circuit: Development, Evolution, and Function
Chairperson: M. Murthy, *Princeton University, New Jersey*

Elkins Memorial Lecture: The Pursuit of Food and Sex: A Study of Olfactory Gain Control
C. Root, *Columbia University, New York*

Neurological Disease Models and Cellular Mechanisms
Chairperson: M. Fortini, *Thomas Jefferson University, Philadelphia, Pennsylvania*

Brain Development and Function
Chairperson: J. Kumar, *Indiana University, Bloomington*

Seymour Benzer Lecture: Control of Dendrite Morphogenesis and Plasticity
Y.-N. Jan, *University of California, San Francisco*

Synaptic Transmission, Development, and Plasticity
Chairperson: K. Ito, *University of Tokyo*

Neural Development and Evolution
Chairperson: I. Salecker, *National Insitute of Medical Research, London, United Kingdom*

Technological Innovations and Applications
Chairperson: R. Mann, *Columbia University, New York*

Poster session

I. Jein, C. Organisti

Cell Death

October 11–15 266 Participants

ARRANGED BY **Douglas Green,** St. Jude Children's Research Hospital
J. Marie Hardwick, Johns Hopkins University
Scott Lowe, Memorial Sloan-Kettering Cancer Center

J.M. Hardwick, S. Lowe, D. Green

The ninth Cold Spring Harbor meeting on cell death was held Tuesday, October 11, through Saturday, October 15. The meeting opened with a keynote presentation from Gerard Evan, who discussed the use of innovative mouse models to understand the role of the MYC oncoprotein in normal tissue homeostasis and cancer. Work from his laboratory clearly highlights the addiction of cancer cells to MYC, whether this is an initiating oncogene or a "client" of other oncogenes such as RAS. MYC inhibition in a variety of cancer models rapidly led to sustained cancer regressions, thereby validating anti-MYC therapies as potentially very effective. In addition to the keynote lecture, there were approximately 60 oral presentations in seven sessions loosely structured around Bcl-2 proteins, Cell Death and Senescence in Cancer, Computational Systems and Approaches, Nonapoptotic Cell Death, Targets and Therapies, New Mechanisms and Components, Mitochondrial Dynamics and Function, and Metabolic Control of Cell Death. Speakers from around the globe included high-profile investigators who were both invited speakers/chairs and abstract presenters. Among these were session chairs David Andrews, Eric Baehrecke, Barbara Conradt, Adi Kimchi, Sally Kornbluth, Anthony Letai, Jennifer Lippincott-Schwartz, Seamus Martin, Huseyin Mehmet, Peter Sorger, Andreas Strasser, Eileen White, Hao Wu, and Junying Yuan, who presented new unpublished data pertaining to a variety of timely topics ranging from new insights into basic apoptotic mechanisms to the identification and preclinical development of new cell death therapies. Beyond this, the meeting was notable for including talks on new approaches in cell death research (for example, computational

C. Crehton, R. Screaton, D. Miller

V. Hindle, C. Bertram, P. Wagner, X. Lowman

methods and functional genomics) and for the large number of presentations related to autophagy and alternative cell death mechanisms. The meeting also had two poster sessions and experimented with a morning poster session format for one of the days. The latter was well attended and considered a success. Finally, Doug Green gave a short address in memory of Jurg Tschopp, a leader in the cell death field who passed away earlier this year. Altogether, much exciting science was presented and the oral sessions were accompanied by lively debate. Although it is clear that the cell death field is burgeoning into many new directions, it is equally apparent that there remain many unanswered questions concerning how cell processes are coordinated and their relevance to disease. Based on the meeting's success and the perceived continuing importance of the field, the meeting will continue in fall 2013 with Scott Lowe, Doug Green, and Sally Kornbluth as organizers.

PROGRAM

Keynote Session
Chairpersons: S. Lowe, *Memorial Sloan-Kettering Cancer Center, New York;* J.M. Hardwick, *Johns Hopkins University, Baltimore, Maryland*

Keynote Address: Finding the Optimal Targets for Effective Cancer Therapy
G.I. Evan, *University of Cambridge, United Kingdom*

BCL-2 Proteins
Chairpersons: D. Andrews, *McMaster University, Hamilton, Canada;* A. Letai, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Cell Death and Senescence in Cancer
Chairpersons: J. Lippincott-Schwartz, *National Institutes of Health, Bethesda, Maryland;* H. Mehmet, *Merck and Co., Rahway, New Jersey*

Computational and Systems Approaches
Chairpersons: P. Sorger, *Harvard Medical School, Boston, Massachusetts;* A. Kimchi, *Weizmann Institute of Science, Rehovot, Israel*

Nonapoptotic Cell Death
Chairpersons: J. Yuan, *Harvard Medical School, Boston, Massachusetts;* E. Baehrecke, *University of Massachusetts Medical School, Worcester*

Targets and Therapies
Chairpersons: A. Strasser, *Walter & Eliza Hall Institute of Medical Research, Parkville, Australia;* E. White, *Rutgers University/The Cancer Institute of New Jersey, New Brunswick*

New Mechanisms and Components
Chairpersons: H. Walczak, *Imperial College, London, United Kingdom;* H. Wu, *Weill Cornell Medical College, New York*

Mitochondrial Dynamics and Function
Chairpersons: D. Green, *St. Jude Children's Research Hospital, Memphis, Tennessee;* B. Conradt, *Ludwig-Maximilians-Universität München, Germany*

Metabolic Control of Cell Death
Chairpersons: S. Kornbluth, *Duke University School of Medicine, Durham, North Carolina;* S. Martin, *Trinity College Dublin, Ireland*

H. Walensky, M. Giam

K. Biachi, P. Meier

Protein Data Bank 40th Anniversary Conference

October 28–30 240 Participants

ORGANIZED BY **Helen Berman,** RCSB PDB, Rutgers University
Stephen K. Burley, Eli Lilly & Co.
Gerard Kleywegt, PDBe, EMBL-EBI, United Kingdom
John Markley, BMRB, University of Wisconsin
Haruki Nakamura, DBj, Osaka University, Japan

A special conference to celebrate the 40th anniversary of the Protein Data Bank (PDB) started at Cold Spring Harbor Laboratory by members attending the X-Ray Methods in Structural Biology course. Several Nobel laureates were in attendance and a Keynote Address was given by Johann Deisenhofer.

The PDB is a repository for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids (see also crystallographic database). The data, typically obtained by X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organizations (PDBe, PDBj, and RCSB). The PDB is overseen by an organization called the Worldwide Protein Data Bank (wwPDB). The PDB is a key resource in areas of structural biology such as structural genomics. Most major scientific journals, and some funding agencies, such as the National Institutes of Health (NIH) in the United States, now require scientists to submit their structure data to the PDB. If the contents of the PDB are thought of as primary data, there are hundreds of derived (i.e., secondary) databases that categorize the data differently. For example, both SCOP and CATH categorize structures according to type of structure and assumed evolutionary relations; GO categorizes structures based on genes.

Session I
M. Rossmann, *Purdue University, West Lafayette, Indiana*
S. Burley, *Eli Lilly & Co., San Diego, California*

Session II
J. Thornton, *Wellcome Trust/EBI, Cambridge, United Kingdom*
D. Baker, *University of Washington, Seattle*
A. Sali, *University of California, San Francisco*

L. Tan, J. Young

S. Bliven, A. Chakrabarty

Session III
J. Richardson, *Duke University, Durham, North Carolina*
A. Bax, *DHHS, NIDDK, LCP, National Institutes of Health, Bethesda, Maryland*
A. Brunger, *HHMI/Stanford University, Stanford, California*

Session IV
C. Arrowsmith, *University of Toronto, Canada*
S. Taylor, *University of California, San Diego*

Session V
S. Wakatsuki, *Structural Biology Research Center, Photon Factory, Japan*
R. Henderson, *MRC Laboratory of Molecular Biology, United Kingdom*
W. Chiu, *Baylor College of Medicine, Houston, Texas*
A. Gronenborn, *University of Pittsburgh, Pennsylvania*
J. Deisenhofer, *University of Texas Southwestern Medical Center, Dallas*

Session VI
K. Wüthrich, *The Scripps Research Institute, La Jolla, California*
M. Hong, *Iowa State University, Ames*
W. Hendrickson, *Columbia University, New York*

Session VII
D. Searls, *Independent Consultant, North Wales, Pennsylvania*


A. McPherson, J. Deisenhofer


W. Hendrickson, R. Henderson


R. Feldman, P. Moore

Genome Informatics

November 1–5 302 Participants

ARRANGED BY **Inanc Birol,** BC Cancer Agency
Jennifer Harrow, Wellcome Trust Sanger Institute
James Taylor, Emory University

This 11th conference was held this year at Cold Spring Harbor Laboratory. The conference series continues to demonstrate a robust attendance, both from the United States and from abroad. It attracted 302 registrants, presenting more than 200 abstracts, and offered a snapshot of the latest developments in the field. There were 11 invited talks and one Keynote Address. The remaining 37 talks were all selected for presentation by session chairs from openly submitted abstracts.

J. Harrow, I. Birol, J. Taylor

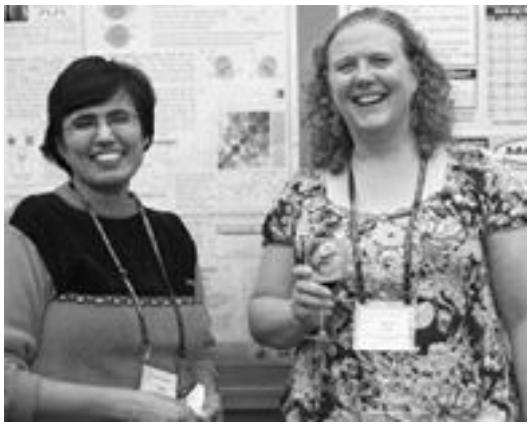
This year, abstracts represented a shifting balance from tool development to applications. Yet the community still agrees that the growth in the capacity of sequencing outpaces the improvements on the informatics side, and there is a continued need to develop scalable analysis tools, with a special emphasis on the reproducibility of their results. The sessions, along with their chairpersons, are listed below.

The Keynote Address, delivered by Dr. Evan E. Eichler, took place before the reception and was the topic of many lively discussions. He challenged researchers to go after the difficult problems in the field and not just concentrate on easy targets. He made his case around structural variations in the repetitive regions of human genomes from diverse populations and primate genomes.

It was encouraging to note that the relative student attendance experienced a peak this year, with 19% of the total registrants. It demonstrates that the field is still growing and attracting new talent across the board.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health.

G. Rätsch, K. Tang



S. Kumari, D. Fisk

PROGRAM

Transcriptomics, Alternative Splicing, and Gene Predictions
Chairpersons: G. Rätsch, *Max-Planck Society, Tübingen, Germany;* S. Jones, *BC Cancer Agency, Vancouver, Canada*

Sequencing Pipelines and Assembly
Chairpersons: Z. Ning, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* M. Schatz, *Cold Spring Harbor Laboratory*

Databases, Data Mining, Visualization, and Curation
Chairpersons: A. Bateman, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* T. Wang, *Washington University, St. Louis, Missouri*

Population and Personal Genomics
Chairpersons: L. Ding, *Washington University School of Medicine, St. Louis, Missouri;* S.C. Sahinalp, *Simon Fraser University, Burnaby, Canada*

Epigenomics and Noncoding Genomes
Chairpersons: A. Milosavljevic, *Baylor College of Medicine, Houston, Texas;* J. Rinn, *Broad Institute of MIT and Harvard University, Cambridge, Massachusetts*

Keynote Address: Genome Structural Variation Discovery and Genotyping
E.E. Eichler, *HHMI/University of Washington*

Comparative and Evolutionary Genomics
Chairpersons: M. Kellis, *Massachusetts Institute of Technology, Cambridge;* S. Batzoglou, *Stanford University, California*

I. Barol, G. Rätsch

S. Goldstein, S. Pepke

Harnessing Immunity to Prevent and Treat Disease

November 16–19 132 Participants

ARRANGED BY **Bali Pulendran,** Emory University
Robert Seder, Vaccine Research Center, NIAID/NIH
Susan Swain, University of Massachusetts Medical School

This meeting covered a broad spectrum of new insights into how the immune system can prevent and treat diseases. This included extensive discussion of strategies to improve T-cell and innate immunity, especially in response to vaccines, with an emphasis on new findings, regulation of innate responses and antigen presentation, and how T cells transition to memory cells with long-lived protective protential.

Stressing the dangers of overexuberant immune responses, the Keynote speaker, Michael Oldstone, introduced the meeting by detailing how antagonists of sphingosine-1 phosphate receptor signaling, involved in lymphocyte motility and migration, could blunt the cytokine storm induced by pathogenic influenza virus infection. B. Rouse later suggested that galectins have an important anti-inflammatory role. The next and several later sessions highlighted the many strategies by which memory T cells protect and how the multiple subsets contribute (S. Swain), as well as the transcriptional regulation of T-cell memory formation in mouse (S. Kaech) and humans (R. Sekaly), providing a deeper understanding of T-cell memory. In related sessions, D. Littman and Y. Belkaid detailed how commensal organisms could profoundly regulate the balance of T-cell subsets in the gut and how the regulatory T cells keep them in check, and L. Hooper discussed how microbiota and host are spatially separated. Several additional talks pointed to organ-specific aspects of immunity (D. Masopust, M. Bogunovic, K. Hofmeyer, S. Turley, and S. Swain).

A major theme was that specific dendritic cells can regulate T cells (B. Pulendran, B. Edelson, and S. Turley), and a discussion of how to develop vaccine approaches to exploit this (R. Seder and A. Hill) was well received. B. Pulendran discussed how the yellow fever vaccine induces the integrated stress response in dendritic cells, thus programming them to induce CD8 T cells. R. Seder discussed the multiple mechanisms by which adjuvants stimulate innate immune receptors, such as Toll-like receptors, to mediate protective immunity against vaccines.

Another theme was the growing recognition that metabolism has a very important role in how immune cells function. E. Pearce discussed metabolic changes in T cells as they differentiate and how these impact their function. In his Keynote talk, Craig Thompson proposed that starvation of cells led to their shedding of all activities except retaining a set of microRNAs that would indicate their identity if they should be rescued.

Welcome: S. Swain, *University of Massachusetts Medical School, Worcester,* and R. Seder, *Vaccine Research Center, NIAID/NIH*

Introduction to Keynote: R. Seder, *Vaccine Research Center, NIAID/NIH*

Keynote Address: Endothelial Cell-Mediated Modulation of Cytokine Storm during Influenza Infection
M. Oldstone, *Scripps Research Institute*

Decision Making in Memory Cell Differentiation
Chairperson: S. Swain, *University of Massachusetts Medical School, Worcester*

Innate Programming of Adaptive Immunity I
Chairpersons: E. Pearce, *Trudeau Institute, Saranac Lake, New York;* D. Lewis, *Stanford University School of Medicine, California*

Innate Programming of Adaptive Immunity II
Chairpersons: L. Haynes, *Trudeau Institute, Saranac Lake, New York;* A. Hill, *Wellcome Trust Centre for Human Genomics, Oxford, United Kingdom*

Organ-Specific Immunity
Chairpersons: S. Turley, *Dana-Farber Cancer Institute, Boston, Massachusetts;* Y. Belkaid, *NIAID, National Institutes of Health, Bethesda, Maryland*

Microbe-Immune Interactions
Chairpersons: L. Hooper, *University of Texas Southwestern Medical Center, Dallas;* D. Littman, *HHMI/New York University School of Medicine, New York*

Introduction to Keynote: S. Swain, *University of Massachusetts Medical School, Worcester*

Keynote Address: How Do Cells Remember?
C. Thompson, *Memorial Sloan-Kettering Cancer Center, New York*

The Journey Within: Human Immunology
Chairpersons: A. Lanzavecchia, *Institute for Research in Biomedicine, Bellinzona, Switzerland;* M. Davis, *HHMI/Stanford University School of Medicine, California*

Plant Genomes and Biotechnology: From Genes to Networks

Nov. 30–Dec. 3 140 Participants

ARRANGED BY **Mary-Lou Guerinot,** Dartmouth College
Todd Mockler, Oregon State University
Detlef Weigel, Max-Planck Institute for Developmental Biology

The theme of the meeting, "Genes, networks, and applications," 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 is also useful for ecological research. The expertise of the three coorganizers spanned the major areas of the meeting, from translational genomics (M.-L. Guerinot, Dartmouth, and S. Briggs, University of California, San Diego) to natural genetic diversity (D. Weigel, Max-Planck Institute for Developmental Biology).

T. Mockler, M.-L. Guerinot, D. Weigel

Following the tradition of the meeting, the two Keynote Addresses, 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. V. Walbot (Stanford) and P. 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 outperform its inbred parents. L. Rieseberg (University of British Columbia), D. Zamir (Hebrew University), D. Weigel, and R. Martienssen (CSHL) 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. J. Ecker (Salk Institute),

D. Caldwell, B. Meyers

K. Creasey, A. Sullivan

S. Hogenout, D. Weigel

B. Gaut (University of California, Irvine), and R. 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 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 P. Benfey (Duke), N. Raikhel (University of California, Riverside), and M.-L. Guerinot. Of course, the genotyping and phenotyping information needs to be captured and interpreted in an integrated fashion. D. Ware (CSHL), J. Carrington (Oregon State), L. Willmitzer (Max-Planck Institute for Molecular Plant Physiology), and T. Mitchell-Olds (Duke) demonstrated different approaches to solving this problem. The ultimate challenge then is to combine these data and to construct genetic, cellular, and physical networks as discussed by J. Traas (ENS Lyon), E. Meyerowitz (Caltech), A. Millar (Edinburgh University), and G. Coruzzi (New York University). The last area is the focus of the National Science Foundation (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 the NSF and several international funding agencies.

As mentioned above, not only are advances in plant biology impacting plant breeding, but they are also important for understanding evolutionary and ecological processes in general, as was discussed by J. Bergelson (University of Chicago) and M. 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.

The meeting was supported by a grant from the NSF and a gift from Dow Agrosciences.

PROGRAM

Introduction to Keynote: M.-L. Guerinot, *Dartmouth*

Keynote Address
O. Leyser, *University of Cambridge*

Phenomics
Chairperson: T. Mockler, *Oregon State University, Corvallis*

Next-Generation Evolutionary Biology
Chairperson: M. Nordborg, *Gregor Mendel Institute, Vienna, Austria*

Next-Generation Plant Breeding
Chairperson: N. Sinha, *University of California, Davis*

New Plant Genomes and Models
Chairperson: U. Kraemer, *Ruhr University Bochum, Germany*

OMICS Integration
Chairperson: T. Michael, *Monsanto, Chesterfield, Missouri*

Next-Generation Plant–Pathogen Interactions
Chairperson: B. Meyers, *University of Delaware, Newark*

Discussion of Current Major Funding Initiatives
Discussion Leader: B. Meyers, *University of Delaware, Newark*

Introduction to Keynote: D. Weigel, *Max-Planck Institute for Developmental Biology*

Keynote Address: Insect Resistance for the Long Haul: Lessons from a Native Plant
I.T. Baldwin, *Max-Planck Institute for Chemical Ecology*

Next-Generation Cell Biology
Chairperson: S. Brady, *University of California, Davis*

RNA and Oligonucleotide Therapeutics

December 4–7 71 Participants

ARRANGED BY **Ryszard Kole,** AVI BioPharma
Adrian Krainer, Cold Spring Harbor Laboratory
Bruce Sullenger, Duke University

This was the first Cold Spring Harbor conference on RNA and Oligonucleotide Therapeutics. The meeting was focused on clinical trials and development of oligonucleotides as drugs, but it brought together top scientists from academia and industry interested in a variety of RNA-based therapeutics, not only oligonucleotides. The theme "From the Bench to the Bedside" made it unique among CSHL meeting series. Covered approaches ranged from antisense oligonucleotides and aptamers through modulation of splicing, targeting expanded triplet repeats and immunostimulation, to small interfering RNAs (siRNAs) and microRNAs (miRNAs) as potential drugs and targets. The all-important issue of RNA therapeutics delivery by a variety of methods was also covered. The applications of these varied technologies included rare diseases, cancer, cardiovascular diseases, and bacterial and viral infections.

The participants came from 11 companies and universities and research institutions from the United States and abroad. The seven scientific sessions featured 37 platform talks and 20 posters and included 90 registered attendees. Animated and insightful exchanges during the sessions continued throughout. A number of participants expressed interest in attending this conference next year.

B. Sullenger, R. Kole, A. Krainer

K. Ryan, D. Seth, Y. Jin, E. Wickstrom

B. Meade, S. Dowdy, H.L. Grimes

PROGRAM

Splice Switching Oligos I
Chairperson: A. Krainer, *Cold Spring Harbor Laboratory*

Splice Switching Oligos II
Chairperson: S. Wilton, *University of Western Australia, Perth, Australia*

Aptamers
Chairperson: P. Giangrande, *University of Iowa, Iowa City*

Delivery
Chairperson: R. Juliano, *University of North Carolina, Chapel Hill*

Antisense and Antagomirs
Chairperson: B. Monia, *Isis Pharmaceuticals, Carlsbad, California*

siRNA/miRNA and Emerging Technologies
Chairperson: A. Khvorova, *RXi Pharmaceuticals, Worcester, Massachusetts*

V. Franciscis, P. Giangrande

S. Yilmaz, E. Petra

L. Hao, L. Jin, A. Oguro

Rat Genomics and Models

December 7–10 134 Participants

ARRANGED BY **Edwin Cuppen,** Hubrecht Institute, the Netherlands
Michael Gould, University of Wisconsin, Madison
Norbert Hubner, Max Delbruck Centrum for Molecular Medicine
Anne Kwitek-Black, University of Iowa

This seventh winter biotechnology conference has been held biannually since 1999 at Cold Spring Harbor Laboratory. It is the first in the United States to focus exclusively on the unique physiological and genetic 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 alternating years, with the most recent meetings being held in Kyoto (2010), Hinxton, UK (2008), Melbourne, Australia (2006), and Kyoto, Japan (2004). Drs. Cuppen, Hubner, Kwitek-Black, and Gould served as conference organizers. The primary goals of this meeting were to (1) promote interactions between biomedical researchers who utilize rat models in the study of physiology, pathophysiology, toxicology, immunology, neuroscience, and oncology and (2) provide an interface between the research community and the various public and commercial entities that support biomedical research in which rat models are used.

N. Hubner, A. Kwitek-Black, M. Gould, E. Cuppen

The meeting was organized into oral presentations by invited speakers; speakers were selected following evaluation of submitted abstracts, poster sessions, and workshops. The Keynote Address was presented by Dr. Qi-Long Ying. The remaining 56 oral presentations were organized into six sessions based on diseases or technologies, and these sessions and their chairpersons are listed on the next page.

Besides the general interactive poster session, where 32 abstracts were presented, and the active participation of 25 young researchers/graduate students, Dr. M. Shimano coordinated a workshop/poster session with 11 abstracts devoted to resources and genomic tools.

J. Coletti, J.A. Landgraf, N. Seiler

C.J. Ma, A. Kwitek

S. Tobias

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 Cold Spring Harbor Laboratory and other sites in the intervening years.

PROGRAM

Genomics, Systems Biology, and Resources
Chairpersons: V. Guryev, *Hubrecht Institute, Utrecht, the Netherlands;* K. Worley, *Baylor College of Medicine, Houston, Texas*

Cardiovascular Diseases I
Chairpersons: S. Cook, *MRC Clinical Sciences Centre, London, United Kingdom;* B. Joe, *Medical College of Ohio, Toledo*

Manipulating the Rat Genome
Chairpersons: Q.-L. Ying, *University of Southern California, Los Angeles;* Z. Izsvak, *Max-Delbrück-Centre, Berlin, Germany*

Keynote Address: Progress in Making Gene Knockout Rats Using ES-Cell-Based Gene-Targeting Technologies
Q.-L. Ying, *University of Southern California, Los Angeles*

Tumor Biology
Chairpersons: J. Shull, *University of Wisconsin, Madison;* B. Smits, *University of Wisconsin, Madison*

Neuroscience and Behavior
Chairperson: E. Cuppen, *Hubrecht Institute, Utrecht, the Netherlands*

Resources Poster/Demo Session

Cardiovascular Diseases II
Chairpersons: A. Kwitek-Black, *University of Iowa, Iowa City;* N. Hubner, *Max Delbrück Centre for Molecular Medicine, Berlin, Germany*

Metabolism and Inflammation
Chairpersons: M. Pravenec, *Academy of Sciences of the Czech Republic, Prague;* T. Aitman, *MRC Clinical Sciences Centre, London, United Kingdom*

ADJUNCT MEETINGS

National Institutes of Health New Investigator Regional Conference

March 14 312 Participants

HOSTED BY **Cold Spring Harbor Laboratory, Office of Sponsored Research**

This special 1-day meeting was specifically designed to educate new investigators on topics such as The Future of Biomedical Research; New and Early-Stage Investigators (an NIH Perspective); and Funding Opportunities, Fostering Success with speakers such as Harold Varmus, Griffin Rodgers, Thomas Insel, Susan Shurin, Sally Rockey, Cheryl Kitt, Samuel Stanley, Jr., and Bruce Stillman. Participants were able to meet in small groups with NIH directors and other NIH representatives (NCI, NIMH, NIAID, NHLBI, NIDDK). An afternoon panel discussion featured "Dos and Don'ts for New Investigators."

Support for this conference was provided by the Alfred P. Sloan Foundation.

PROGRAM

The future of biomedical research and the fate of new investigators
B. Stillman, *Cold Spring Harbor Laboratory*

New and early-stage investigators: An NIH perspective
S. Rockey, *NIH Office Extramural Research*

Funding Opportunities and Challenges for New Investigators
T. Insel, *National Institute of Mental Health*
G. Rodgers, *National Institute of Diabetes and Digestive and Kidney Diseases*
S. Shurin, *National Heart Lung and Blood Institute*
H. Varmus, *National Cancer Institute*

The Peer Review Process: From Application to Funding of a New Investigator
C.I. Kitt, *NIH Center for Scientific Review*

NIH Peer Review Dos and Don'ts for New Investigators
K. Collins, *University of California, Berkeley*
H. Yeh, *Dartmouth University*
S. Hammes, *University of Rochester*
S. Stanley, Jr., *Stony Brook University*

Fostering the Success of New Investigators
S. Rockey, *NIH Office of Extramural Research*

Closing Remarks

New York Structural Biology Group Summer Session

August 1 274 Participants

ORGANIZED BY **David Cowburn,** Albert Einstein College of Medicine
David Eliezer, Weill Medical College of Cornell University
Hao Wu, Weill Medical College of Cornell University

This 1-day discussion group provided a forum for structural biology in the New York area. The speakers and their presentations are listed below.

This meeting was cosponsored by the National Science Foundation (NSF) Research Coordination Network. Other sponsors included Bristol-Myers Squibb, GE Healthcare, Nature Structural & Molecular Biology, Gatan, GenScript, TTP Labtech, Rigaku FEI Company, Cell Press, ImClone Systems, Inc., JEOL, Varian Roche, Bruker Biospin, and Wyatt Technology.

PROGRAM

Structures of the bacterial ribosome in classical and hybrid states of tRNA binding
S. Blanchard, *Weill Cornell Medical College*

Coupling RNAi to heterochromatin: Insights into the structure of the RITS complex
T. Schalch, *Cold Spring Harbor Laboratory*

Tracing a killer's path: Structural insights into the activation pathway of pro-apoptotic BAX
E. Gavathiotis, *Albert Einstein College of Medicine*

Tracking death domain complex assembly with NMR
P. Driscoll, *MRC National Institute for Medical Research, London*

Multiscale simulation of cadherin-mediated cell adhesion
Y. Wu, *Columbia University*

Structural studies of human GABA(B) receptor.
Q. Fan, *Columbia University*

Using SPR to study interactions of membrane proteins
B. Lang, *GE Healthcare*

Structural biology of the human cancer protein interaction network
G. Montelione, *Rutgers University*

DNMT1 and maintenance DNA methylation.
J. Song, *Memorial Sloan-Kettering Medical Institute*

Signal transduction in RAGE: Structural biology of the receptor for advanced glycation end products
A. Shekhtman, *SUNY, Albany*

Conserved RNA recognition element identifies ZBP1-operated posttranscriptional regulatory networks
V. Patel, *Albert Einstein College of Medicine*

Structural analysis of SLAC1-family anion channel activity
W. Hendrickson, *Columbia University*

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.

Workshop on Leading Bioscience

February 18–21

ARRANGED BY **C. Cohen,** Newton, Massachusetts
D. Kennedy, Cambridge, Massachusetts

Bioscience now dominates the academic sciences, and the 21st century is often heralded as the century in which the life sciences and biotechnologies will have their greatest impact on society. Life science expertise is now relevant in disciplines from the environment to energy, from psychology to forensics. Many biologists seek research careers in academia or industry, whereas others explore more varied careers in education, publishing, business, or law. Few life scientists experience formal leadership training to facilitate their development as future leaders in their chosen profession. The first in a series of intensive workshops focusing on Leadership in Biosciences emphasized learning by

doing and involved role playing, giving and receiving feedback, and group problem-solving. Much of the learning was peer-to-peer. It was an intensive 3 1/2-day event where almost 30 participants learned to develop the skills to lead and interact effectively with others in both one-on-one and group settings.

This workshop was generously supported with funds provided by American Express Philanthropy.

PARTICIPANTS

Akakura, S., Ph.D., Roswell Park Cancer Institute, Buffalo, New York
Bethke, A., Ph.D., Boyce Thompson Institute/Cornell University, Ithaca, New York
Chi, H., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Du, Y.-C.N., Ph.D., Weill Cornell Medical College, New York
Flames, N., Ph.D., Columbia University, New York
Fuchs, S., Ph.D., University of North Carolina, Chapel Hill, North Carolina
Hjerling Leffler, J., Ph.D., New York University Medical School, New York
Huss, W., Ph.D., Roswell Park Cancer Institute, Buffalo, New York
Kadiri, L., Ph.D., Cold Spring Harbor Laboratory
Madzima, T., Ph.D., Florida State University, Tallahassee
Manjarrez Orduno, N., Ph.D., The Feinstein Institute, Manhasset, New York
Neher, S., Ph.D., University of North Carolina, Chapel Hill
Oh, S.W., Ph.D., Allen Institute for Brain Science, Seattle, Washington
Petrella, L., Ph.D., University of California, Santa Cruz
Premsriut, P., Ph.D., Cold Spring Harbor Laboratory
Salvador Esteban, B., Ph.D., University of San Francisco, California
Schwertassek, U., Ph.D., Cold Spring Harbor Laboratory
Smith, B., Ph.D., Arizona State University, Tempe
Truscott, M., Ph.D., University of Illinois, Chicago
Ubeda, C., Postdoc, Memorial Sloan-Kettering Institute, New York
Wheeler, T., Ph.D., University of Rochester, New York
Wood, D., Harvard Medical School/BIDMC, Boston, Massachusetts
Zhang, J., Ph.D., University of Alabama, Birmingham

SEMINARS

Khan, Z., The Rockefeller University, New York: Strategy and evaluation.
Miller, K., Brown University, Providence, Rhode Island: Science in the public eye.

Protein Purification and Characterization

April 6–19

INSTRUCTORS

R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, M.D. Anderson Cancer Center/University of Texas, Houston
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
A. Esch, University of Wisconsin, Madison
P. Kuhn, University of Wisconsin, Madison
P. Kwong, University of California, Los Angeles
Y.-U. Lee, M.D. Anderson Cancer Center, University of Texas, Houston, Texas
X. Li, M.D. Anderson Cancer Center/University of Texas, Houston, Texas
C. Olson, Brandeis University, Waltham, Massachusetts
K. Pennington, Brandeis University, Waltham, Massachusetts
A. Ponce, University of California, Los Angeles
N. Thompson, University of Wisconsin, Madison

This course was for scientists who were not familiar with techniques of protein isolation and characterization. It is a rigorous program that included laboratory work all day and a lecture with 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 including 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 broadly and to cancer research in particular.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Charles, R., M.D, Massachusetts General Hospital, Boston
Hawwari, A., Ph.D., King Faisal Specialist Hospital & Research Center, Riyadh
Hevener, K., Ph.D., University of Illinois at Chicago
Keeler, A., B.S., University of Iowa, Iowa City
Lyssenko, N., Ph.D., Children's Hospital of Philadelphia, Pennsylvania
Marada, S., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Mochalkin, I., Ph.D., EMD Serono Research Institute, Billerica, Massachusetts
Morgan, J., B.S., Stanford Research USD, Sioux Falls, South Dakota
Patterson, K., Ph.D., The University of Texas at Austin
Skeie, J., Ph.D., University of Iowa, Iowa City
Stutz, A., M.Sc., University of Massachusetts Medical School, Worcester
Termathe, M., M.A., Max-Planck Institute for Molecular Biomedicine, Munster
Tia, S., B.S., University of California, Berkeley
Wang, P., B.A., Harvard University, Boston, Massachusetts
Yu, Y., Ph.D., Cold Spring Harbor Laboratory
Zhang, S., B.S., University of Pennsylvania, Philadelphia

SEMINARS

Burgess, R., University of Wisconsin, Madison: Welcome and introduction to course. Introduction to protein purification. Protein purification in the mid-60s: RNA polymerase and sigma factor. Rapid isolation of weakly binding proteins with the IFAST system.
Chumanov, R., University of Wisconsin, Madison/Promega: Halo tag and purification of CARM1.
Courey, A., University of California, Los Angeles: System-wide analyses of Groucho and SUMO in *Drosophila* development.
Lin, S.-H., MD Anderson Cancer Center, Houston, Texas: Cell adhesion molecules and prostate cancer metastasis.
Marr, M., Brandeis University, Waltham, Massachusetts: Transcriptional and translational cellular responses to stress.
Thompson, N., University of Wisconsin, Madison: MAbs and immunoaffinity chromatography in cancer research.

Cell and Developmental Biology of *Xenopus*

April 8–19

INSTRUCTORS **A. Sater,** University of Houston, Texas
G. Thomsen, Stony Brook University, New York

ASSISTANTS **B. Dzamba,** University of Virginia, Charlottesville
T. Nakayama, University of Virginia, Charlottesville
K. Pfister, University of Virginia, Charlottesville

Xenopus is a leading vertebrate model for the study of gene function in development. The combination of lineage analysis, gene-knockout strategies, experimental manipulation of the embryo, and genomic/bioinformatic techniques makes it ideal for studies on the molecular control of embryo patterning, morphogenesis. and organogenesis. The course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *Xenopus* development. An important element was the informal interaction between students and course faculty.

Technologies covered included oocyte and embryos, analysis of limb and tail regeneration in tadpoles, time-lapse imaging of morphogenesis, gain- and loss-of-function analyses using mRNAs and antisense oligos, whole-mount in situ hybridization, immunocytochemistry, microsurgery, and genomic/bioinformatic techniques, preparation of transgenic embryos, and use of *Xenopus tropicalis* for genetic analyses. This course was designed for those new to the *Xenopus* field, as well as for those wanting a refresher course in the emerging technologies. The course was open to investigators from all countries.

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

Alvarado Kristensson, A., Ph.D., Lund University, Malmoe, Sweden
Chernet, B., B.S., Tufts University, Medford, Massachusetts
Feric, M., B.S., Princeton University, New Jersey
Getwan, M., Dipl., Institute of Zoology, Stuttgart, Germany
Hatch, V., B.Sc. University of East Anglia, Norwich, United Kingdom
Ioannou, A., B.S., University of Cyprus, Nicosia
Pai, Vaibhav, V., Ph.D., Tufts University, Medford, Massachusetts
Sedzinski, J., M.A., Max-Planck Institute, Dresden, Germany
Strobl, A.-C., M.S., National Institute of Medical Research, London, United Kingdom
Travis, S., Ph.D., University of Iowa, Iowa City
Wang, W.-L., Ph.D., Albert Einstein College, Bronx, New York
Watson, A., B.A., MRC National Institute for Medical Research, London, United Kingdom
Wylie, L., B.S., University of Cambridge, United Kingdom
Yim, D., B.Sc., Duke-NUS Graduate Medical School/TUMCB, Singapore
Zimmerman, L., Ph.D., National Institute for Medical Research, London, United Kingdom

SEMINARS

Gilchrist, M., National Institutes for Medical Research, London, United Kingdom: *Xenopus*: genomes, genomics, and computational biology. Workshop: Online resources for *Xenopus* gene expression data.
Gingeras, T., Cold Spring Harbor Laboratory: Biological lessons learned about the human and fly transcriptomes from the looking at the numbers.
Grainger, R., University of Virginia, Charlottesville: Workshop. Genomic approaches to enhancer identification. Analysis of eye formation in *Xenopus*: Model system for studies of determination and organogenesis.
Houston, D., University of Iowa, Iowa City: Symmetry breaking in the Xenopus egg-localized RNAs set the stage.
Keller, R., University of Virginia, Charlottesville: Morphogenesis.
Khokha, M., Yale University School of Medicine, New Haven, Connecticut: Introduction to *X. tropicalis* and using *Xenopus* to study human disease genes.
Kroll, K., Washington University School of Medicine, St. Louis, Missouri: Epigenetic regulation of early cell-fate acquisition.
Lin, K., University of Minnesota, Minneapolis: Cellular and molecular mechanisms of appendage regeneration in *Xenopus laevis*.
Lowe, S., Cold Spring Harbor Laboratory: Identifying tumor suppressor and tumor maintenance genes using mouse models and RNAi.
Nakayama, T., University of Virginia, Charlottesville: Approaches for manipulating gene expression in *Xenopus*.
Sater, A., University of Houston, Texas: *Xenopus* as a model system: History and prospects.
Thomsen, G., Stony Brook University, New York: The Xenopus Organizer.
Wallingford, J., University of Texas, Austin: The awesome power of live imaging in *Xenopus*.

Workshop on Autism Spectrum Disorders

June 8–15

INSTRUCTORS **D. Geschwind,** University of California, Los Angeles
P. Levitt, University of Southern California, Los Angeles
S. Spence, Children's Hospital, Boston, Massachusetts

ASSISTANT **K. Aldinger,** University of Southern 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. Both neurological and medical conditions often occur in the disorder. The underlying etiology remains a mystery, but ASD is one of 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, 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, but it also introduced them to many potential collaborators working to understand ASD from different disciplines. The workshop had hands-on exercises to complement the featured intense lecture sessions.

PARTICIPANTS

Acharya, V., M.D., Penn State University, Hershey
Aida, T., Ph.D., Tokyo Medical and Dental University, Japan
Atbasoglu, E.C., Ankara University, Turkey
Aykan, S., B.S., Ankara University, Turkey
Bangash, M.A., B.S., Johns Hopkins School of Medicine, Baltimore, Maryland
Belgard, T., B.S., National Institutes of Health, Bethesda, Maryland
Carrulo, M.P., Ph.D., Children's Hospital, Boston & Harvard University, Boston, Massachusetts
Glavis-Bloom, C., Ph.D., SRI International, Menlo Park, California
Gordon, I., Yale University, New Haven, Connecticut
Grubb, M., B.S., New York University, New York
Jung, J.-Y., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Lippman Bell, J., Ph.D., Children's Hospital, Boston/Harvard University, Boston, Massachusetts
Liu, X., B.S., The University of Tokyo, Japan
Lucchina, L., B.S., IFIBYNE-CONICENT, Buenos Aires, Argentina
McEvoy, K., B.S., University of California, Los Angeles
Mehta, S., B.S., University of California, Los Angeles
Nakai, N., B.S., Hiroshima University, Japan
Raznahan, A., Ph.D., National Institutes of Mental Health, NIH, Bethesda, Maryland
Soke, G.N., B.S., University of Colorado/JFK Partners, Denver

SEMINARS

Biederer, T., Yale University, New Haven, Connecticut: Synaptic differentiation and autism spectrum disorders.
Geschwind, D. University of California, Los Angeles: Introduction to human genetics and findings in ASDs. Genomics/gene expression. Molecular models of ASD in humans.
Hensch,T., Harvard University, Boston, Massachusetts: Critical periods/plasticity mechanisms.
Kasari, C., University of California, Los Angeles: Treatments overview.
Levitt, P., University of Southern California, Los Angles: Mammalian animal models. Neuroanatomical systems implicated in ASD and neuropathology.
Martinez, F., University of Arizona, Tucson: Environmental contributions: Asthma as a model.
McPartland, J., Yale University, New Haven, Connecticut: Neurocognition and face processing mechanisms.
McPartland, J. and Pelfrey, K., Yale University, New Haven, Connecticut: Introduction to imaging and ERP.
Newschaffer, C., Drexel University, Philadelphia, Pennsylvania: Epidemiology of ASD: Are rates really rising—Can we even tell?
Pardo-Villamizar, C., Johns Hopkins University School of Medicine, Baltimore, Maryland: Neuroimmunology.
Pelfrey, K., Yale University, New Haven, Connecticut: Neuroimaging findings in ASD.
Rapin, I., Albert Einstein School of Medicine, Bronx, New York: Autism's odyssey—1943–2011.
Sahin, M., Children's Hospital, Boston, Massachusetts: Translational trials: Rapamycin in tuberous sclerosis complex.
Spence, S., Children's Hospital, Boston, Massachusetts: Clinical assessments (clinical videos, student excercises: ADI-R, ADOS, SRS). Medical models/comorbidities. Endophenotypes, language delay, epilepsy/regression, OFC.
Stone, W., University of Washington, Seattle: Clinical presentation, core features. ASD measures, broader phenotypes, phenotypic variability, outcomes.
Suomi, S., NICHD, National Institutes of Health, Bethesda, Maryland: Evolutionary perspectives on social behavior.
Szyf, M., McGill University, Montreal, Canada: Epigenetics.
Wall, D., Harvard University, Cambridge, Massachusetts: Genomic tools exercises: Bioformalities, database access, data gathering and uses.

Advanced Bacterial Genetics

June 8–28

INSTRUCTORS
D. Hughes, Uppsala University, Sweden
B. Lazazzera, University of California, Los Angeles
F. Yildiz, University of California, Santa Cruz

ASSISTANTS
J. Bergman, Uppsala University, Sweden
S. Hoover, University of California, Los Angeles
N. Shikuma, University of California, Santa Cruz

This course presented logic and method used in the genetic dissection of complex biological processes in diverse bacteria. Laboratory methods included classical mutagenesis using transposons, mutator strains, and chemical mutagens; recombineering with single- and double-stranded DNA; detection of gene expression changes using various reporter genes; the mapping of mutations using genetic and physical techniques; modern approaches to the generation and analysis of targeted gene disruptions and fusions using polymerase chain reaction (PCR) and cloning methods; epitope insertion mutagenesis; site-directed mutagenesis; and fluorescence microscopy. Key components of the course were the use of sophisticated genetic methods in the analysis of model bacterial (including *Escherichia coli, Salmonella, Bacillus subtilis*, and *Vibrio cholerae*) and the use of the wealth of new genomic sequence information to motivate these methods.

Invited lecturers presented various genetic approaches to study bacterial mechanisms of metabolism, development, and pathogenesis.

PARTICIPANTS

Anderson, E., B.S., Whitehead, Institute/MIT, Cambridge, Massachusetts
Ballok, A., B.S., Dartmouth Medical School, Hanover, New Hampshire
Cenens, W., B.Sc., KuLeuven, Heverlee, Belgium
Currie, D., B.S., Dartmouth College MCB Program, Hanover, New Hampshire
Engstrom, A., M.Sc., Swedish Institute for Infectious Disease Control, Solna, Sweden
Hatosy, S., University of California, Irvine
Hsin, J., Ph.D., Stanford University, California
Jochumsen, N., B.S., Technical University of Denmark, Kgs. Lynbgy, Denmark
Kubik, T., B.Sc., The Rockefeller University, New York
Mathis, R., M.S., ETH Zurich, Duebendorf, Switzerland
Moolman, M., M.Sc., Delft University of Technology, Delft, The Netherlands
Neupane, M., D.V.M., Michigan State University, Lansing
Rivera, A., Ph.D., University of Illinois, Urbana-Champaign
Schiessl, K., Dipl., Eawag, Dubendorf, Switzerland
Zhang, H., Ph.D., University of Central Florida, Orlando
Ziegler, S., Dipl., Albert-Ludwigs University, Freiburg, Germany

SEMINARS

DiRita, V., University of Michigan Medical School, Ann Arbor: Genetic approaches for discovering genes and uncovering mechanisms in *Vibrio cholera* and *Campylobacter jejuni*.
Ibba, M., Ohio State University, Columbus: Aminoacyl-tRNA synthetases and translation quality control.
Levin, P., Washington University, St. Louis, Missouri: A nucleotide sugar coordinates cell size with carbon availability in divergent model organisms.
Maloy, S., San Diego State University, California: Plasticity of the bacterial genome.
Matic, I., INSERM, Paris, France: Modulation of mutation rates in bacteria.
Sawitzske, J., National Cancer Institute, Frederick, Maryland: Optimization of oligo recombination and an update on the molecular mechanisms.
Visick, K., Loyola University Medical Center, Maywood, Illinois: Use of genetics to dissect the complex regulatory pathway controlling biofilm formation and colonization by *Vibrio fischeri*.
Winkler, M., Indiana University, Bloomington: Cell wall biosynthesis and antibiotic stress responses in pneumococcal growth and virulence.

Ion Channel Physiology

June 8–28

INSTRUCTORS
N. Golding, The University of Texas, Austin
P. Kammermeier, University of Rochester Medical Center, New York
M. Nolan, University of Edinburgh, United Kingdom

ASSISTANTS
C. Bladen, University of Calgary, Alberta, Canada
A. Fink, Columbia University, New York
A. Graves, Northwestern University, Evanston, Illinois
K. Hansen, Emory University School of Medicine, Atlanta, Georgia
L. Kelly, The Rockefeller University, New York
V. Lu, NIH/NIAAA/LMP, Rockville, Maryland
K. Ogden, Emory University, Atlanta, Georgia
M. Roberts, The University of Texas at Austin
C. Schoonover, Columbia University, New York

The primary goal of this course was to investigate, through lectures and laboratory work, the properties of ion channels that allow neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) respond to neuromodulators with changes in functional properties, or (4) are developmentally required and regulated by light. The research interests of guest lecturers reflected these areas of emphasis.

The laboratory component of the course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice prepa-

rations. 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 was 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

Abraira, V., Ph.D., Johns Hopkins Medical Institute, Baltimore, Maryland
Agosto, M., Ph.D., Baylor College of Medicine, Houston, Texas
Buffington, S., B.S., Baylor College of Medicine, Houston, Texas
Che, Y., B.S., University of Connecticut, Storrs
Christianson, J., Ph.D., University of Colorado, Boulder
Hennessey, J., B.A., Duke University, Durham, North Carolina
Kirkby, L., M.Physicology, University of California, Berkeley
Nagaraja, C., BEME, Uppsala University, Sweden
Rosen, Z., B.A., Columbia University, New York
Sitaraman, D., Ph.D., Yale University, New Haven, Connecticut
Stempel, V., B.Sc., Charite Berlin, Germany
Wang, G., B.S., Boston University, Massachusetts

SEMINARS

Brenowitz, S., NIDCD/National Institutes of Health, Bethesda, Maryland: EPSCs.
Brown, D., University College London, United Kingdom: Evening research lecture.
Clark, B., University College London, United Kingdom: Sodium channels.
Colecraft, H., Columbia University, New York: Calcium channels.
Deisseroth, K., Stanford University, California: Channel engineering 1.
Du Lac, S., The Salk Institute, La Jolla, California: Intrinsic plasticity, microcircuits.
Farrant, M., University College London, United Kingdom: Synapses, GABA/IPSCs.
Kramer, R., University of California, Berkeley: Channel engineering 2.
Larsoon, P., University of Miami, Florida: Potassium channels.
Schwartz, K., National Institutes of Health, Bethesda, Maryland: Voltage-gated ion channels."
Sjostrom, J., University College London, United Kingdom: Plasticity.
Spruston, N., Northwestern University, Evanston, Illinois: Synaptic integration and dendrites."
Traynelis, S., Emory University, Atlanta, Georgia: Ligand-gated channels; glutamate receptors.
Tsien, R., Stanford University Medical Center, California

Molecular Embryology of the Mouse

June 8–28

INSTRUCTORS	**K. Hadjantonakis,** Memorial Sloan-Kettering Cancer Center, New York **J. Rivera-Perez,** University of Massachusetts Medical School, Worcester
CO-INSTRUCTORS	**R. Johnson,** MD Anderson Cancer Center, Houston, Texas **X. Sun,** University of Wisconsin, Madison
ASSOCIATE CO-INSTRUCTOR	**D. Escalante-Alcalde,** Institute of Cellular Physiology-UNAM, Mexico
ASSISTANTS	**E. Domyan,** University of Wisconsin, Madison **J. Herriges,** University of Wisconsin, Madison **T. Huang,** University Medical School, Worcester, Massachusetts **S. Munoz-Descalzo,** University of Cambridge, Cambridge, United Kingdom **Y. Yoon,** University of Massachusetts Medical School, Worcester

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 stem cell biology, embryogenesis, and organogenesis, whereas laboratory practicals provided extensive hands-on introductions to mouse stem cell and embryo analysis. Experimental techniques covered included in vitro culture and manipulation of pre- and postimplan-

tation embryos, embryo transfer, culture and derivation and genetic manipulation of embryonic stem (ES) cells, production of chimeras by embryo aggregation and by ES cell injection, and transgenesis by pronuclear microinjection. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including techniques of histology, in situ hybridization, immunohistochemistry, skeletal preparation, organ culture, and tissue recombination. 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

Buecker, C., M.Sc., Stanford School of Medicine, California
Carrera, I.-M., Ph.D., Ebiotec Foundation, La Coruna, Spain
Chojnowski, J., Ph.D., University of Georgia, Athens
Cinalli, R., B.S., New York University School of Medicine, New York
Colaneri, A., Ph.D., National Institute of Environmental Health Sciences, RTP, North Carolina
Davis, F., Ph.D., Northeastern University, Boston, Massachusetts
Gundry, R., Ph.D., Medical College of Wisconsin, Milwaukee
Hannibal, R., Ph.D., Stanford University, California
Huang, A., Ph.D., Shriners Hospital for Children Research Center, Portland, Oregon
Menshykau, D., M.S., ETH Zurich, Basel, Switzerland
Norville, J., Ph.D., Massachusetts Institute of Technology, Cambridge
O'Brien, K., Ph.D., Cornell University, Ithaca, New York
Ouspenskaia, T., B.S., The Rockefeller University, New York
Steidl, C., M.D., British Columbia Cancer Agency, Vancouver, Canada

SEMINARS

Behringer, R., The University of Texas/MD Anderson Cancer Center, Houston: Transgenesis.
Capel, B., Duke University Medical Center, Durham, North Carolina: Germ cells and development.
Foley, A., Weill Cornell Medical College, New York: Cardiovascular development.
Hadjantonakis, K., Memorial Sloan-Kettering Cancer Center, New York: Mouse embryo imaging: Confocal approaches.
Johnson, R., Anderson Cancer Center, Houston, Texas: Somitogenesis. Liver development and hippo signaling.
Joyner, A., Memorial Sloan-Kettering Cancer Center, New York: Patterning and genetic fate mapping approaches: Application in the nervous system.
Justice, M., Baylor College of Medicine, Houston, Texas: ENU mutagenesis.
Lovell-Badge, R., MRC National Institute for Medical Research, London, United Kingdom: Sex determination.
Magnuson, T., University of North Carolina, Chapel Hill: Epigenetics.
McMahon, A., Harvard University, Cambridge, Massachusetts: Kidney development.
Mills, A., Cold Spring Harbor Laboratory: Chromosome engineering.
Nagy, A., Samuel Lunenfeld Research Institute, Toronto, Ontario, Canada: iPS cells.
Pevny, L., University of North Carolina, Chapel Hill: Retinogenesis and eye development.
Rivera-Perez, J., University of Massachusetts Medical School, Worcester: Early postimplantation development: Morphogenesis and cell lineage.
Robertson, E., University of Oxford, United Kingdom: Signaling in the early postimplantation embryo and axial development.
Rossant, J., The Hospital for Sick Children, Toronto, Ontario, Canada: Early cell lineages, embryo-derived stem cells, and chimeras.
Shen, M., Columbia University Medical Center, New York: Prostate development, stem cells, and cancer.
Solter, D., Institute of Medical Biology, Singapore: Historical overview of the mouse as a model system. Preimplantation development.
Soriano, P., Mt. Sinai School of Medicine, New York: Gene targeting, gene traps and recombinases: Theory and applications.
Sun, X., University of Wisconsin, Madison: Limb development. Lung development.
Sussel, L., Columbia University, New York: Pancreas development.
Tam, P., Children's Medical Research Institute, Westmead, Sydney, Australia: Gastrulation and establishment of the germ layers.
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: Craniofacial development and the neural crest.
Turnbull, D., New York University Medical Center, New York: Mouse embryo imaging: Ultrasound and MRI.

Proteomics

June 13–28

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
CO-INSTRUCTOR	**M. Cilia,** USDA-ARS, Ithaca, New York
ASSISTANTS	**S. DeBlasio,** Cold Spring Harbor Laboratory **B. King,** Cornell University, Ithaca, New York **C. Ruse,** Cold Spring Harbor Laboratory **P. Samir,** Vanderbilt University, Nashville, Tennessee **B. Weindorf,** Cold Spring Harbor Laboratory **C. Zheng,** University of Washington, Seattle

This intensive laboratory and lecture course focused on cutting-edge proteomic approaches and technologies. Students gained practical experience purifying and identifying protein complexes and posttranslational modifications. In a section focused on quantitative whole-proteome analyses or top-down proteomics, students gained hands-on experience using two-dimensional gel electrophoresis and mass spectrometry analysis. Students used differential in-gel electrophoresis (DIGE) for gel-based protein quantification. Differentially expressed proteins were recognized by statistical methods using advanced gel analysis software and identified using MALDI mass spectrometry. For shotgun proteomic analysis sections or bottom-up proteomics, students used label-free and covalent isotopic-labeling quantitative approaches to differentially profile changes in protein complexes and whole proteomes. Students were trained in high-sensitivity microcapillary liquid chromatography coupled with nanospray-ESI and tandem mass spectrometry analysis. Students learned both single-dimension and multidimensional separation methods. In a section focused on targeted proteomics, students learned to analyze and process shotgun proteomic data for the development of SRM/MRM assays

that accurately identify and quantify targeted proteins. Students designed transitions for selected peptides and performed SRM/MRM mass spectrometry assays. They learned to process and interpret the acquired data to measure changing quantities of targeted proteins in a variety of biological samples. For all sections of the course, a strong emphasis was placed on data analysis. A series of outside lecturers discussed various proteomics topics including imaging by mass spectrometry, de novo sequence analysis, advanced mass spectrometry methods, protein arrays, and functional proteomics. The aim of the course was to provide each student with the fundamental knowledge and hands-on experience necessary for performing and analyzing proteomic experiments. The overall goal was to train students to identify new opportunities and applications for proteomic approaches in their biological research.

This course is supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Bangasser, D., Ph.D., The Children's Hospital of Philadelphia, Pennsylvania
Hinton, S., Ph.D., College of William and Mary, Williamsburg, Virginia
Mancewicz Arslanoglu, J., M.S., Metropolitan Museum of Art, New York
Mesrobian, H., M.S., Medical College of Wisconsin, Milwaukee
Messner, D., Ph.D., Bastyr University, Kenmore, Washington
Morgenstern, D., B.Sc., University of Queensland, St. Lucia, Australia
Poulsen, L., Ph.D., Technical University of Denmark, Kgs. Lyngby, Denmark
Ralston, K., Ph.D., University of Virginia, Charlottesville
Rowland, S., B.S., University of Maryland/IMET, Baltimore, Maryland
Silva, G., M.S., New York University, New York
Singh, R., M.Sc., Florida State University, Tallahassee
Slavoff, S., Ph.D., Harvard University, Cambridge, Massachusetts
Subramanian, V., Ph.D., Whitehead Institute, Cambridge, Massachusetts
Taka, E., Ph.D., Florida A&M University, Tallahassee
Taylor, H., Ph.D., Meharry Medical College, Nashville, Tennessee
Waldron, L., B.S., University of North Carolina, Chapel Hill
Zhou, S., Ph.D., Tennessee State University, Nashville

SEMINARS

Andacht, T., Centers for Disease Control and Prevention, Atlanta, Georgia: Fundamentals of 2-D DIGE for top-down proteome analysis.
Beckett, P., General Electric, Piscataway, New Jersey: Application of Decyder software to analyze and interpret 2-D DIGE for top-down proteome analysis.
Bruce, J., University of Washington, Seattle: Fundamentals of mass spectrometry for proteomics.
Bulman, A. Bruker Daltonics, Billerica, Massachusetts: Introduction and application of MALDI-TOF/TOF mass spectrometry in proteomics.
Chait, B., The Rockefeller University, New York: Proteomics analysis of proteins complexes and protein machines: Viral infections and nuclear pore complex.
Chaurand, P., University of Montreal, Canada: Tissue imaging and profiling by mass spectrometry.
Clauser, K., Broad Institute of MIT/Harvard, Cambridge, Massachusetts: Manual de novo peptide MS/MS interpretation for evaluating database search results.
Cristea, I., Princeton University, New Jersey: A proteomics perspective of the dynamic interplay between oncogenic viruses and hosts.
Eng, J., University of Washington, Seattle: MS/MS sequence database searching and downstream analysis.
Friedman, D., Vanderbilt University, Nashville, Tennessee: Variation and experimental design for proteomic analyses, Variation and experimental design for proteomic analyses: 2D-DIGE and LC-MS/MS proteomics.
Hunt, D., University of Virginia, Charlottesville: Application electron transfer dissociation for the analysis of peptides and posttranslation moc. Identification of cancer therapeutics derived from dysregulated signal transduction pathways.
Kellie, J., Northwestern University, Evanston, Illinois: Methods and applications for top down proteomics. Top-down analysis of mass spectrometry from intact and fragmented proteins.
Link, A., Vanderbilt University, Nashville, Tennessee: Introduction and overview of the 2011 Cold Spring Harbor Laboratory Proteomics Course.
Pappin, D., Cold Spring Harbor Laboratory: Fundamentals of mass spectrometry to identify peptides and proteins. Fundamentals of quantitative mass spectrometry for proteomics.
Smolka, M., Cornell University, Ithaca, New York: Investigating cell signaling networks with quantitative proteomics.

Workshop on Pancreatic Cancer

June 16–June 22

INSTRUCTORS **D. Bar-Sagi,** New York University Medical Center, New York
A. Maitra, Johns Hopkins University School of Medicine, Baltimore, Maryland
D. Tuveson, Cambridge Research Institute, United Kingdom

ASSISTANT **K. Olive,** Columbia University, New York

Pancreatic cancer is one of the deadliest cancers: Tumors are often diagnosed at advanced stages of the disease and metastasize rapidly. This 1-week discussion course provided a comprehensive overview of clinical and biological aspects of pancreatic cancer with special emphasis on disease diagnosis and management, molecular pathways involved in tumor development and progression, mechanism-based therapeutic strategies, advanced research tools, and ethical concerns. Attendees were able to interact with senior investigators on a one-to-one basis in an informal environment. Nonfaculty students were eligible for partial stipends, depending on stated need. Applications were invited from medical and graduate students, postdoctoral fellows, and faculty.

PARTICIPANTS

Aizenberg, R., B.S., Pancreatic Cancer Action Network, Manhattan Beach, California
Bednar, F., Ph.D., University of Michigan, Ann Arbor
Collins, M., B.S., University of Michigan, Ann Arbor
Court, H., Ph.D., New York University Medical Center, New York
DelGiorno, K., B.S., SUNY Stony Brook, New York
Drosos, Y., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Goni, E., B.S., University Vita-Salute San Raffaele, Milano, Italy
Holland, A., B.S., Monash University, Clayton, Australia

Holmstrom, S., Ph.D., University of Texas Southwestern, Dallas
Huang, L., Ph.D., Ontario Cancer Institute, University of Toronto, Canada
Ludwig, M., B.S., University of Alabama at Birmingham
Morris I.V., J., B.S., University of California, San FranciscoPiciucchi, M., M.D., Sapienza University II Medical School, Rome, Italy
Roy, I., B.S., Medical College of Wisconsin, Milwaukee
Schmerr, M., Ph.D., Cleveland Clinic, Ohio
Solar, M., B.S., Hospital Clinic/IDIBAPS, Barcelona, Spain
Tsai, S., M.D., Medical College of Wisconsin, Milwaukee
Wang, M., Ph.D., University of Texas Southwestern Medical Center, Dallas
Westphalen, C., Ph.D., Columbia University Medical Center, New York

SEMINARS

Bar-Sagi, D., New York University Medical Center, New York: Pathways.
Der, C., University of North Carolina, Chapel Hill: Target identification.
Garrett-Mayer, E., Medical University of South Carolina, Clarkson: Clinical trial design.
Goggins, M., Johns Hopkins Medical School, Baltimore, Maryland: Molecular genetics.
Hahn, W., Dana Farber Cancer Institute/Harvard Medical School, Boston, Massachusetts: Target identification.
Hebrok, M., University of California, San Francisco: Pathways.
Hollingsworth, M., University of Nebraska Medical Center, Omaha: Resources and career development.
Kelly, K., University of Virginia, Charlottesville: Imaging new modalities: Mouse.
Kindler, H., University of Chicago Medical Center, Illinois: Clinical trial design.
Longnecker, D., Dartmouth Medical School, Hanover, New Hampshire: Anatomy and physiology.
Maitra, A., Johns Hopkins University School of Medicine, Baltimore, Maryland: Histopathology.
Megibow, A., New York University, New York: Diagnosis and imaging II.
Olive, K., Columbia University, New York: Microenvironment: Nonimmune.
Pandey, A., Johns Hopkins Medical School, Baltimore, Maryland: Omics: Proteomics.
Pollack, J., Stanford University, California: Molecular genetics.
Simeone, D., University of Michigan Medical Center, Ann Arbor: Diagnosis and imaging I.
Spalholz, B., National Cancer Institute, Rockville, Maryland: Resources and career development.
Tempero, M., University of California, San Francisco: Cachexia and pain.
Tuveson, D., Cambridge Research Institute, United Kingdom: Mouse models.
Van der Heiden, M., Massachusetts Institute of Technology, Cambridge: Omics: Metabolomics.
Vonderheide, R., University of Pennsylvania, Philadelphia: Microenvironment: Immune.
Wu, A., University of California, Los Angeles: Imaging new modalities: Human.
Welch, J., National Institutes of Health, Baltimore, Maryland: Clinical trial design.

Molecular Neurology and Neuropathology

June 27–July 3

INSTRUCTORS **M. Cookson,** National Institute on Aging/NIH, Baltimore, Maryland
B. Davidson, University of Iowa College of Medicine, ,[Need city]
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 such as protein aggregation, specific vulnerability of certain classes of neuron, and long incubation period and discussed to what extent these features reflect common pathological mechanisms. The course further explored how the underlying mechanisms in these disparate disorders may be targeted for potential diagnostic and therapeutic gain. Extended seminars and discussion by a wide range of leading investigators further illuminated developments in this rapidly moving field. Participation in the course provided an essential conceptual and methodological framework for anyone intending to pursue rigorous research.

The course was held at the Laboratory's Banbury Conference Center located on the north shore of Long Island. All participants stayed within walking distance of the Center, close to a tennis court, pool, and private beach. Participants were encouraged to arrive in the afternoon of June 26 for an evening start.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Chua, J., B.S., University of Michigan, Ann Arbor
Gogliotti, R., B.S., Northwestern University, Chicago, Illinois
Hauser, D., B.S., NIH/National Institutes on Aging, Bethesda, Maryland
Henderson, M., Ph.D., National Institute on Drug Abuse, Baltimore, Maryland
Hill, J., B.S., University of Texas Medical School at Houston, Houston
Ingram, M., B.S., University of Minnesota, Minneapolis
Jorgensen, N., B.S., University of Copenhagen, Copenhagen, Denmark
Kay, V., B.S., University London College Institute of Neurology, United Kingdom
Korff, A., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Machamer, J., Ph.D., Johns Hopkins University, Baltimore, Maryland
Neuhuber, B., B.S., National Institutes of Health, North Bethesda, Maryland
Peelaerts, W., B.S., Laboratory for Neurobiology and Gene Therapy, Leuven, Belgium
Pradhan, S., B.S., Stanford University, California
Sassi, C., M.D., NIH/National Institutes on Aging/University College London, Bethesda, Maryland
Van der Kant, R., B.S., Netherlands Cancer Institute, Amsterdam, The Netherlands
VanGilder, R., Ph.D., West Virginia University, Morgantown
Vannini, E., B.S., Scuola Normale Superiore, C.N.R., Pisa, Italy

SEMINARS

Cookson, M., NIH/National Institute on Aging, Baltimore, Maryland: Parkinson's disease.
Darnell, J., The Rockefeller University, New York: Special lecture.
Davidson, B., University of Iowa, Ames: Lysosmal diseases.
Duff, K., Colombia University, New York: Alzheimer's disease.
Iadecola, C., Weill Cornell Medical Center, New York: Stroke.
McNamara, J., Duke University Medical Center, Durham, North Carolina: Epilepsy.
Morris, H., Cardiff University, United Kingdom: Neurological aspects.
Nelson, D., Baylor College of Medicine, Houston, Texas: Fragile X and associated syndromes.
Orr, H., University of Minnesota, Minneapolis: Spinocerebellar ataxia.
Rademakers, R., Mayo Clinic College of Medicine, Jacksonville, Florida: Front temporal dementia.
Rothstein, J., Johns Hopkins University School of Medicine, Baltimore, Maryland: Amyotrophic lateral sclerosis.
Warren, S., Emory University School of Medicine, Atlanta, Georgia: Schizophrenia.

Computational Cell Biology

July 1–21

INSTRUCTORS
L. Loew, University of Connecticut Health Center, Farmington
G. Smith, College of William and Mary, Williamsburg, Virginia
J. Tyson, VirginiaTech, Blacksburg

ASSISTANTS
A. Ahmet, Colgate University, Hamilton, New York
S.-A. Brown, University of Connecticut Health Center, Farmington
C. Chen, Virginia Tech, Blacksburg
M. LaMar, The College of William and Mary, Williamsburg, Virginia
C. Oguz, Virginia Tech, Blacksburg
O. Walch, The College of William and Mary, Williamsburg, Virginia

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 is focused on simulation of the molecular machinery (genes–proteins–metabolites) that underlie the physiological behavior (input-output characteristics) of living cells.

This 3-week course incorporated a series of didactic lectures on the mathematics of dynamical systems, computational simulation techniques, cell biology, and molecular biology. Practicing theoreticians and experimentalists rotated in for 1–3-day visits during the course to give lectures and interact with the students. Midway through the course, students selected an area for independent study, and the focus of the last week of the course was largely on these projects, supplemented by continued visiting lecturers.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Bush, A., M.Sc., Universidad de Buenos Aires, Argentina
Carta, A., M.S., INRIA, Sophia Antipolis Cedex, France
Gao, M., B.S., Vanderbilt University, Nashville, Tennessee
Gomez, J., B.S., University of Iowa, Iowa City
Grzenda, A., Mayo Clinic, Rochester, Minnesota
Jones, Z., B.S., Middle Tennessee State University, Murfreesboro
Joshi, A., M.Tech, Intelligent Systems Research Centre, University of Ulster, Londonderry, United Kingdom
Kuhn, H., B.S., Harvard University, Boston, Massachusetts
Lu, Z., B.S., University of Texas Health Science Center, San Antonio
Marchchenko, O., B.A., University of Connecticut Health Center, Farmington
O'Donoghue, D., B.Sc., University College London, United Kingdom
Parker, K., B.S., Moss Landing Marine Labs, Los Altos, California
Paulson, J., B.S., University of Maryland, College Park
Qin, H., Ph.D., Spelman College, Atlanta, Georgia
Song, J., Ph.D., New Mexico State University, Las Cruces
Sun, G., B.S., Johns Hopkins University, Baltimore, Maryland
Troaca-Luchici., L.-A., B.Eng., King's College London, United Kingdom
Wang, X., B.S., The College of William and Mary, Williamsburg, Virginia

SEMINARS

Albert, R., Penn State University, University Park: Discrete dynamic modeling of signal transduction networks.
Blackwell, A., George Mason University Krasnow Institute, Fairfax, Virginia: Neuronal signaling.
Blackwell, A., George Mason University Krasnow Institute, Fairfax, Virginia; Keener, J., University of Utah, Salt Lake City; Smith, G., The College of William and Mary, Williamsburg, Virginia; Moraru, I., University of Connecticut Health Center, Farmington; and Schaff, J., University of Connecticut Health Center, Farmington: Exercises.
Edelstein-Keshet, L., University of British Columbia, Vancouver: Building up a model from the group up. A case study using a simple epidemic model.
Faeder, J., University of Pittsburgh, Pennsylvania: Rule-based modeling.
Hasty, J., University of California, San Diego, La Jolla: Gene networks.
Hucka, M., California Institute of Technology, Pasadena: SMBL, model databases, and translation.
Iyengar, R., Mount Sinai School of Medicine, New York: Signaling models.
Keener, J., University of Utah, Salt Lake City: Cardiac physiology.
Keener, J., University of Utah, Salt Lake City, and Smith, G., The College of William and Mary, Williamsburg, Virginia: NeuroRD/Cardiac models/Stochastic models.
LaMar, D., and Day, S., The College of William and Mary, Williamsburg, Virginia: Network topology/chemical reaction network theory.
Lechleiter, J., University of Texas Health Sciences Center, San Antonio: Calcium signaling.
Loew, L., University of Connecticut Health Center, Farmington: Model building w/VCell; VCell and ODEs.
Ma'ayan, A., Mt. Sinai School of Medicine, New York: Network analysis in systems biology.
Mendes, P., University of Manchester, United Kingdom: Copasi and parameter estimation.
Moraru, I., and Loew, L., University of Connecticut Health Center, Farmington: VCell and ODEs.
Moraru, I., and Schaff, J., University of Connecticut Health Center, Farmington: VCell stochastic and spatial modeling.
Phair, R., Integrative Bioinformatics, Los Altos, California: Molecular cell biology and ODEs, fluorescent proteins, and tracer kinetics.
Schaff, J., and Loew, L., University of Connecticut Health Center, Farmington: VCell and PDEs; actin dendritic nucleation.
Schaff, J., Loew, L., and Moraru, I., University of Connecticut Health Center, Farmington: VCell spatial exercises.
Sherman, A., National Institutes of Health, Bethesda, Maryland: Modeling calcium dynamics.
Smith, G., The College of William and Mary, Williamsburg, Virginia: Cellular biophysics and ODEs. Random walks and diffusion. Stochastic modeling.
Terman, D., Ohio State University, Columbus: Dynamical systems. Introduction to XPP. Waves. XPP exercises.
Tyson, J., Virginia Tech, Blacksburg: How cells compute/network motifs and functional modules. Temporal organization of the cell cycle/design principles of biochemical oscilliators. XPP exercises.
Wellman, H., Mathworks, Natick, Massachusetts: Matlab.
Wolf, V., Saarland University, Germany: Stochastic modeling of biochemical networks.
You, L., Duke University, Durham, North Carolina: Modeling and experimental analysis of mammalian cell cycle entry. Programming bacteria.

Frontiers and Techniques in Plant Science

July 1–21

INSTRUCTORS
S. Harmer, University of California, Davis
R. Last, Michigan State University, E. Lansing
J. Maloof, University of California, Davis

ASSISTANTS
Y. (Polly) Hsu, University of California, Davis
J. Kim, Michigan State University, E. Lansing
O. Wilkins, Boston, Massachusetts

This course provided an intensive overview of topics in plant physiology, biochemistry, and development, focusing on genomic, analytical, computational, and other high-throughput approaches to understanding plant biology. It emphasized recent results from model organisms including *Arabidopsis*, maize, and tomato, as well as a variety of other plants, and provided an introduction to current methods used in basic and applied plant biology. It was designed for scientists with some experience in molecular techniques or in plant biology who wish to work with plants using the latest molecular, genomic, and computational technologies. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. The instructors and a stellar group of invited speakers, acknowledged leaders in their fields, presented up-to-the-moment research on a wide range of topics in plant research.

These seminars included plant evolution, morphology, and anatomy; various topics in plant development (including development of meristems, gametophytes, and roots); light perception and photomorphogenesis; cell wall biosynthesis and biofuels, function, and perception of hormones; small RNAs; biotic and abiotic interactions; and applications addressing 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 expert overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided exposure to cutting-edge techniques currently used in plant research. These included studies of plant devel-

opment and genome evolution, transient gene expression, applications of fluorescent proteins, automated phenotyping, analysis of polysomal mRNA, analysis of global gene expression data (microarray and short-read sequencing), construction of genetic networks, and metabolome analysis. Students were introduced to leading computational environments and programs including R, Bioconductor, and ImageJ. 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

DeVries, A., B.S., University of California, Riverside
Diaz, J., B.S., University of California, Riverside
Dorn, K., B.S., University of Minnesota, St. Paul
Hillmer, R., S.M., University of Minnesota, St. Paul
Jorgensen, M.E., University of Copenhagen, Denmark
Katifori, E., Ph.D., The Rockefeller University, New York
Lee, I., Ph.D., Yonsei University, Seoul, South Korea
Norville, J., Ph.D., Massachusetts Institute of Technology, Cambridge
Ricigliano, V., B.A., St. Johns University, Queens, New York
Rogers, E., B.S., University of Illinois, Chicago
Sanabria, N., Ph.D., University of Johannesburg, South Africa
Sanchez, P., Ph.D., The University of Arizona, Tucson
Seidl-Adams, I., Ph.D., The Pennsylvania State University, University Park
Sparks, E., B.S., Vanderbilt University, Nashville, Tennessee
Weaver, J., B.S., Northwestern University, Boston, Massachusetts
Weng, J., B.A., Dartmouth, Hanover, New Hampshire

SEMINARS

Bailey-Serres, J., University of California, Riverside: Plant stress responses.
Beachy, R., Donald Danforth Plant Science Center, St. Louis, Missouri: Opportunities and challenges in basic and applied plant biology and agriculture.
Bomblies, K., Harvard University, Cambridge, Massachusetts: Speciation.
Brady, S., University of California, Davis: Root growth and development.
Brutnell, T., Boyce Thompson Institute, Ithaca, New York: Plastids.
Harmer, S., University of California, Davis: Circadian rhythms.
Hollick, J., University of California, Berkeley: Maize genetics.
Jackson, D., Cold Spring Harbor Laboratory: Shoot development.
Kellogg, E., University of Missouri, St. Louis: Phylogenetics. Sequence analysis and phylogeny reconstruction.
Kuhlemeier, C., University of Bern, Switzerland: Phyllotaxis.
Juntawong, P., University of California, Riverside: Quantitative PCR.
Last, R., Michigan State University, E. Lansing: Secondary metabolites.
Maloof, J., University of California, Davis: Quantitative genetics. QTL analysis.
Mayfield, S., University of California, San Diego, La Jolla: Algal biofuels.
McSteen, P., University of Missouri, Columbia: Auxiliary meristems.
Miller, N., University of Wisconsin, Madison: Phenotyping using machine vision.
Montgomery, B., Michigan State University, E. Lansing: Light signaling.
Nemhauser, J., University of Washington, Seattle: Hormone signaling.
Poethig, S., University of Pennsylvania, Philadelphia: Introduction to plant anatomical development.
Preuss, S., Monsanto Company, Chesterfield, Missouri: Stress responses.
Roossnick, M., Noble Foundation, Oklahoma: Plant/virus interactions.
Schilmiller, A., Michigan State University, E. Lansing: Analysis of plant volatiles.
Shiu, S.-H., Michigan State University, E. Lansing: Genome evolution.
Spalding, E., University of Wisconsin, Madison: Plant growth.
Timmerman, M., Cold Spring Harbor Laboratory: RNA interference.
Walling, L., University of California, Riverside: Plant/pest interactions.
Wang, L., Boyce Thompson Institute, Ithaca, New York: RNA-Seq.
Zheng, N., University of Washington, Seattle: F-box signaling.

Neurobiology of *Drosophila*

July 1–21

INSTRUCTORS
H. Broihier, Case Western Reserve University, Cleveland, Ohio
D. Featherstone, University of Illinois, Chicago
A. Rothenfluh, University of Texas Southwestern Medical Center, Dallas

ASSISTANTS
T. Fergestad, University of Wisconsin, Madison
R. James, Case Western Reserve University, Cleveland, Ohio
O. Khorramshahi, FU Berlin, Germany
E. Knoche, Charite Berlin, Germany
S. Ojelade, University of Texas Southwestern Medical Center, Dallas
D. Owald, Charite Berlin, Germany

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The 3-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that were used in the investigation of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization, labeling of identified neurons, electrophysiology (intracellular recording and patch clamping), optical imaging of neuronal activity, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system.

This course was supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders and Stroke, and the Howard Hughes Medical Institute.

PARTICIPANTS

Aimon, S., B.S., Institut Curie, Paris, France
Erion, R., B.S., University of Pennsylvania, Philadelphia
Etchegary, J., B.A., Boston University, Massachusetts
Franconville, R., Ph.D., Howard Hughes Medical Institute, Ashburn, Virginia
Herbert, S., B.A., Champalimaud Neuroscience Programme, Lisbon, Portugal
Johri, S., M.Sc., University of Utah, Salt Lake City
Kochinke, K., B.S., Radboud University, Nijmegen, The Netherlands
Martelli, C., Ph.D., Yale University, New Haven, Connecticut
Mishra, B., Ph.D., University of Michigan, Ann Arbor
Piccioli, Z., Ph.D., Massachusetts Institute of Technology, Cambridge
Velazquez, U.-N., Ph.D., University of California, San Francisco
Wang, Q.-P., Ph.D., Garvan Institute of Medical Research, Darlinghurst, Australia

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: Axon pathfinding.
Broihier, H., Case Western University, Cleveland, Ohio, and Lee, C.-H., NICHD/National Institutes of Health, Bethesda, Maryland: Genetic screens.
DiAntonio, A., Washington University, Seattle: The axonal injury response lessons from flies and mice.
Dubnau, J., Cold Spring Harbor Laboratory: Learning and memory.
Featherstone, D., University of Illinois, Chicago: Ephys introduction.
Freeman, M., University of Massachusetts, Worcester: Glia. Bioinformatics/Web tools.
Galko, M., University of Texas/MD Anderson, Houston: Nocioception. Neuroanatomy.
Kravitz, E., Harvard Medical School, Boston, Massachusetts: Aggression.
Lee, C.-H., NICHD/National Institutes of Health, Bethesda, Maryland: Visual system development.
Levitan, E., University of Pittsburgh, Pennsylvania: Imaging, peptide release/dense core vesicle trafficking.
Murthy, M., Princeton University, New Jersey: CNS function.
O'Connor-Giles, K., University of Wisconsin, Madison: NMJ structure and development.
Rolls, M., Penn State University, University Park: Neuronal cell biology.
Rothenfluh, A., University of Texas Southwestern Medical Center, Dallas: Drugs.
Ruta, V., Columbia University, New York: CNS functional organization.
Turner, G., Cold Spring Harbor Laboratory: CNS processing.
Verstreken, P., Katholieke Universiteit Leuven, Belgium: Imaging; Peptidegric transmission.
Vosshall, L., The Rockefeller University, New York: Feeding.
White, B., NIMH/National Institutes of Health, Bethesda, Maryland: Tools for neuronal function and circuitry.
Zhang, B., University of Oklahoma, Norman: Genetic techniques/FINGR.

Structure Function and Development of the Visual System

July 6–19

INSTRUCTORS **B. Chapman,** University of California, Davis
W.M. Usrey, 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 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; anatomy, physiology, and perceptual significance of parallel pathways; functional parcellation of extrastriate cortex; role of patterned neuronal activity in the development of central visual pathways; and molecular mechanisms of development and plasticity in the visual system.

PARTICIPANTS

Allegra, M., B.S., Scuola Normale Superiore di Pisa, Italy
Barone, I., B.S., CNR Neuroscience Institute, Pisa, Italy
Davis, Z., B.S., University of California Neuroscience Graduate Group, Davis
Dillingham, C., B.S., Cardiff University, United Kingdom
Dubis, A., B.S., Medical College of Wisconsin, Milwaukee
Gilbert, M., B.S., University of California, San Diego, La Jolla
Greenwald, S., B.S., University of Washington, Seattle
Inagaki, M., Ph.D., Osaka University, Toyonaka, Japan
Kiley, C., B.S., University of California, Davis
Kim, E., Ph.D., University of California, San Diego, La Jolla
Lankow, B., B.S., University of California, Davis

Lewis, C., B.S., Ernst Strüngmann Institute, Frankfurt am Main, Germany
Lim, H., Ph.D., Hunter College of the City of New York University, New York
Reitsma, D., B.S., The Medical College of Wisconsin, Milwaukee
Short (Hembrook), J., B.S., University of New Hampshire, Durham
Solomon, S., B.S., University of Sydney, Australia
Stewart, E., B.S., University of Adelaide, Australia
Williams, K., B.S., McMaster University, Hamilton, Canada

SEMINARS

Chapman, B., University of California, Davis: How the visual system got its stripes.
Chichilnisky, E., Salk Institute, La Jolla, California: Functional organization of the retina.
Connor, E., Johns Hopkins University, Baltimore, Maryland: Neural coding of object structure.
Dickson, K., *Cell Press*, Cambridge, Massachusetts: Career guidance.
Feldheim, D., University of California, Santa Cruz: Mechanisms of topographic mapping.
Feller, M., University of California, Berkeley: Mechanisms and development of retinal direction selectivity.
Fitzpatrick, D., Max-Planck Florida Institute, Jupiter: What maps tell us about the structure, function, and development of circuits in visual cortex.
Gallant, J., University of California, Berkeley: Representation of natural scenes and movies: Evidence from neurophysiology and fMRI.
Guido, W., Virginia Commonwealth University, Alexandria: Circuit development in the dorsal lateral geniculate nucleus of the mouse.
Hirsch, J., University of Southern California, Los Angeles: Neural circuits for visual processing in thalamus.
Horton, J., University of California, San Francisco: Functional architecture of striate cortex.
Huberman, A., University of California, San Diego: Genetic approaches to parsing visual circuit development, function, and regeneration.
Krauzlis, R., Salk Institute, La Jolla, California: Eye movement: Physiology.
Martin, K., University of Zurich, Switzerland: Visions of Johanna.
Movshon, A., New York University, New York: Brain mechanisms of visual motion perception.
Neitz, J., The Medical College of Wisconsin, Milwaukee: Cones, color vision, and the outer retina.
Nawy, S., Albert Einstein College of Medicine, Bronx, New York: Synaptic origins of parallel visual pathways in primate.
Sherman, M., University of Chicago, Illinois: Considerations of thalamocortical and corticothalamic relationships.
Stone, L., NASA Ames Research Center, Moffett Field, California: Eye movement: Perception and reality.
Usrey, M., University of California, Davis: Dynamic interactions between thalamus and cortex for visual processing.
Usrey, M. and Chapman, B., University of California, Davis: The basics. Career guidance.
Wandell, B., Stanford University, Connecticut: Human visual field maps: Where they are and how they respond. Seeing words: Cortical responses and white matter pathways.

Advanced Techniques in Molecular Neuroscience

July 6–21

INSTRUCTORS	**C. Lai,** Indiana University, Bloomington **R. Lansford,** California Institute of Technology, Pasadena
ASSOCIATE INSTRUCTORS	**T. Bell,** NDRI Resource, Philadephila, Pennsylvania **L. Fenno,** Stanford University, California **K. Haas,** University of British Columbia, Vancouver, Canada
TEACHING ASSISTANTS	**D. Bower,** California Institute of Technology, Pasadena **C. Canaria,** California Institute of Technology, Pasadena **F. Chen,** University of Connecticut, Storrs **R. Draft,** Harvard University, Cambridge, Massachusetts **M. Girgenti,** University of Connecticut, Storrs **L. Gunaydin,** Stanford University, California **D. Huss,** California Institute of Technology, Pasadena **B. Maher,** University of Connecticut, Storrs **E. Munroe,** Indiana University, Bloomington **K. Podorski,** University of British Columbia, Vancouver, Canada **E. Robinson,** Indiana University, Bloomington

This laboratory and lecture course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises, daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory, and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and served to illustrate the ways in which the various experimental approaches have been used to advance specific areas of neurobiology. In this year's course, the laboratory portion included topics such as an introduction to the design and use of animal virus vectors in neurobiology; the use of small interfering RNAs (siRNAs) for regulating the expression of specific genes in neurons; practical exercises in gene delivery systems, including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use, and design of 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 (RT)-PCR (polymerase chain reaction) analyses from small numbers of cells (RNA purification, PCR optimization, interpretation of results); single-cell PCR and cDNA library construction; and methods and application of RNA amplification (aRNA). Each laboratory module was followed by comprehensive data analyses and interpretation, protocol troubleshooting, and suggestions for ways to improve or modify the existing technique. Finally, course participants were introduced to bioinformatics and a wide range of Internet resources available to molecular neuroscientists.

The course was generously supported by New England BioLabs Inc.; Olympus America Inc.; Carl Zeiss Inc. along with Bitplane-Andor Technology; Bio-Rad Laboratories; Chroma Technology Corporation; Coherent Laser; Eppendorf North America; Fotodyne Inc.; Harvard Apparatus Inc.; Illumina Inc.; Invitrogen Corporation; Leica Microsystems Inc.; Nanodrop Technologies; Narishige International USA Inc.; PerkinElmer Life; Promega Corporation; Qiagen Inc.; Roche Applied Science; Sigma-Aldrich; and Thermo Fisher Scientific. This course was also 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

Ashby, R., Ph.D., Australian National University, Canberra, Australia
Balu, D., Ph.D., McLean Hospital/Harvard Medical School, Belmont, Massachusetts
Brosius Lutz, A., Ph.D., Stanford University, California
Chen, Y., Ph.D., Max-Planck Institute for Medical Research, Heidelberg, Germany
Dougherty, K., Ph.D., Karolinska Institute, Stockholm, Sweden
Fournier, N., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Fricks-Gleason, A., Ph.D., University of Utah, Salt Lake City
Gao, W.-J., Ph.D., Drexel University College of Medical, Philadelphia, Pennsylvania
Godfrey, B., Ph.D., Makerere University College of Health Sciences, Kampala, Uganda
Gundlfinger, A., Ph.D., Brain Research Institute, University of Zurich, Switzerland
London, S., Ph.D., University of Illinois, Urbana-Champaign
Morgan, J., Ph.D., University of California, Davis School of Medicine, Sacramento
Skene, N., Ph.D., University of Cambridge, Hinxton, United Kingdom
Sitko, A., B.S., Columbia University, New York
Vajn, K., M.D., University of Pittsburgh, Pennsylvania
Whitney, I., B.S., University of California, Santa Barbara

SEMINARS

Barres, B., Stanford University School of Medicine, California: What do astrocytes do?
Bell, T., University of Pennsylvania, Philadelphia: Single-neuron mRNA analysis. Optogenetics module. *Xenopus* neural transfection module. In utero RNAi module.
Darnell, R., HHMI/The Rockefeller University, New York. Maps of the RNA world.
DiAntonio, A., Washington University School of Medicine, St. Louis, Missouri: The axonal injury response: Lessons from flies and worms.

Dymecki, S., Harvard Medical School, Boston, Massachusetts: Decoding the mouse serotonergic system using intersectional genetics and selective in vivo silencing.

Eberwine, J., University of Pennsylvania Medical School, Philadelphia: The secret life of a single cell.

Gunaydin, L., Stanford University, California: Single neuron mRNA analysis. Optogenetics module. *Xenopus* neural transfection module.

Haas, K., University of British Columbia, Vancouver, Canada: Single neuron mRNA analysis. Optogenetics module. *Xenopus* neural transfection module.

Lai, C., Indiana University, Bloomington: Cloning and lentivirus module. BAC recombination module. Neural imaging module. In utero RNAi module. Lentivirus and imaging module.

Lansford, R., California Institute of Technology, Pasadena: Cloning and lentivirus module. BAC recombination module. Neural imaging module. Cloning and lentivirus module. In utero RNAi module. Lentivirus and imaging module. In utero RNAi module.

Lichtman, J., Harvard University, Cambridge, Massachusetts: Talking about science. Neural imaging module. Cloning and lentivirus module. BAC recombination module. Lentivirus and imaging module. In utero RNAi module. Imaging the connectome.

Lledo, P.-M., Pasteur Institute, Paris, France: Adult-born neurons and brain plasticity.

Loturco, J., University of Connecticut, Storrs: Neural imaging module. Cloning and lentivirus module. BAC recombination module. In utero RNAi module. Single-neuron mRNA analysis.

Distinguished Alumni Lecture: Storey, K., University of Dundee College of Life Science, United Kingdom: Mechanisms regulating neural differentiation.

Circuits and Connectivity in the Vertebrate Brain

July 6–21

INSTRUCTORS

P. Mitra, Cold Spring Harbor Laboratory
K. Rockland, RIKEN-MIT Center for Neural Circuits Genetics, Cambridge, Massachusetts
M. Witter, Norwegian University of Science and Technology, Trodheim, Norway

ASSISTANTS

S. Mukhopadhyay, Cold Spring Harbor Laboratory
K. O'Reilly, Norwegian University of Science and Technology, Trodheim, Norway
S. Ozen, Stanford University, San Francisco, California
V. Pinskly, Cold Spring Harbor Laboratory
N. Wall, Salk Institute for Biological Studies, La Jolla, California

In comparison with complete reference genomes now available for multiple species, our knowledge about the neuronal and circuit architecture of vertebrate nervous systems is relatively sparse. However, this situation is rapidly changing, enabled by technical advances as well as resurgent and widespread interest in the neuroscientific community in mapping out neural circuitry at unprecedented scales, ranging from the reconstruction of local microcircuits to the mapping of brain-wide mesocircuits. This circuit architecture naturally and logically complements the molecular architecture as delineated by the mapping of brain-wide gene expression patterns. Experimental efforts are under way in multiple species, promising to advance our knowledge of the wiring logic of the vertebrate brain. This will fundamentally impact our understanding of brain function and evolution and also have an essential role in understanding pathological changes in circuitry that underlie neurological and neuropsychiatric disorders.

This 2-week workshop brought together classical neuroanatomical approaches along with the new techniques that are enabling a new generation of neuroanatomical research into the circuit and

molecular architecture of the vertebrate brain. The workshop had three main components: classical, molecular, and computational neuroanatomy. An experimental component of the workshop involved injection-based tract tracing in the mouse, using classical and viral tracer substances in wild-type and transgenic mice. Lectures covered classical (tracer injections, sectioning, histochemistry, imaging) and molecular (genetic engineering of mice as well as viral tracers and optogenetic probing of circuits) techniques. Material was presented by simultaneous viewing of slides under light microscopy as well as digital images, including an in-depth orientation to Internet resources. The computational component involved hands-on algorithmic analysis and interpretation of digital neuroanatomical data sets, from both electron microscopy and light microscopy. Species covered included rodents and human and nonhuman primates, with special lectures on other vertebrate lineages. One lecture addressed the invertebrate brain.

This meeting was sponsored by Howard Hughes Medical Institute and Cold Spring Harbor Laboratory.

PARTICIPANTS

Bienkowski, M., B.S., Center for Neuroscience, University of Pittsburgh, Pennsylvania
Chung, S., Ph.D., University of California, Berkeley
Dittrich, L., Ph.D., SRI International, Menlo Park, California
Engerer, K., B.S., Medical College of Wisconsin, Milwaukee
Gauthier, J., Ph.D., Princeton University, New Jersey
Karnani, M., Ph.D., University of Cambridge, United Kingdom
Keeley, P., B.S., University of California, Santa Barbara
Martinez-Molina, N., M.Sc., Instituto de Neurosciencas, San Juan de Alicante, Spain
Matho, K., B.S., Institut de la Vision, Paris, France
Schreiweis, C., M.A., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Viswanathan, S., B.S., HHMI/Janelia Farms Research Campus, Ashburn, Virginia
Yanik, M., B.S., Massachusetts Institute of Technology, Cambridge

SEMINARS

Amunts, K., Institute of Neuroscience and Medicine, Juelich, Germany: Human cerebral cortex: Cytoarchitectonic parcellation and non-DTI fib tracking.
Barbas, H., Boston University School of Medicine, Massachusetts: Sensory pathways and emotion.
Bohland, J., Boston University, Massachusetts: An overview of image analysis challenges in high-throughput neuroanatomy.
Bota, M., University of Southern California, Los Angeles: Data and knowledge integration across multiple levels of the mammalian CNS.
Burkhalter, A., Washington University, St. Louis, Missouri: Network of mouse visual cortex.
Enquist, L., Princeton University, New Jersey: Transneuronal spread of α herpes virus infection using PRV to trace neural circuits.
Grange, P., Cold Spring Harbor Laboratory: Computational techniques for the analysis of gene-expression atlases.
Hawrylycz, M., Allen Institute for Brain Science, Seattle, Washington: Computational neuroanatomy of gene expression.
Helmstaedter, M., Max-Planck Institute for Medical Records, Heidelberg, Germany: Dense circuits reconstruction from blockface electron microscopy data.
Huang, J., Cold Spring Harbor Laboratory: Genetic dissection of GABAergic circuits in cerebral cortex.
Karten, H., University of California, San Diego, LaJolla: Natural selection is a tinkerer: Evolutionary origins of neocortex. Conservation of microcircuitry.
Kleinfeld, D., University of California, San Diego, La Jolla: Feed forward versus cyclic connectivity. The confluence of reafference and exafference. Basic principles of light microscopy.
Lee, J.-H., University of California, Los Angeles: Optogenetic stimulation and fMRI.
Li, B., Cold Spring Harbor Laboratory: Habenula in psychiatric disorders.
Martin, K., University of Zurich, Switzerland: Mapping the matrix.
Mello, C., Oregon Health and Science University, Portland: Gene expression in zebra finch.
Mitra, P., Cold Spring Harbor Laboratory: Course introduction.
Osten, P., Cold Spring Harbor Laboratory: Antomy and c-*fos*-based activation mapping by serial two-photon (STP) tomography.
Peterson, D., Rosalind Franklin University, N. Chicago, Illinois: Quantitative histology: Introduction to design-based stereology.
Rockland, K., Masschusetts Institute of Technology/RIKEN Brain Science Institute, Cambridge: Introduction to experimental techniques: Axon transport, tracers and connections.

Rosa, M., Monash University, Victoria, Australia: Toward a computational atlas of the cerebral cortex and its connections.

Safdieh, J., Weill Cornell Medical College, New York: General organization of the human central and peripheral nervous system and case studies.

Saper, C., Beth Israel Deaconess Medical Center, Boston, Massachusetts: Hypothalamic regulation of sleep and circadian rhythms.

Schiff, N., Cornell University Medical College, New York: Arousal regulation in intact forebrain.

Sharfman, H., New York University Langone Medical Center, Orangeburg: Hippocampal anatomy and physiology under normal and pathological conditions.

Strausfeld, N., University of Arizona, Tucson: Conventional and neurophylogenetic studies of neural circuits. Part I: Circuit cracking: The conventional route. Part II: What 600 million years of evolution tells us.

Ugurbil, K., University of Minnesota, Minneapolis: Human brain imaging, from cortical columns and layers to networks.

Witter, Menno, Norwegian University of Science and Technology, Trodheim, Norway: Functional neuroanatomy to study the entorhinal-hippocampal network.

Zaborsky, L., Rutgers University, Newark, New Jersey: Extracting structural and functional information from large- and small-scale reconstructions of the basal forebrain cholinergic system.

Zilles, K., Institute of Neuroscience and Medicine, Juelich, Germany: Human cerebral cortex: Transmitter receptors, functional segregation, and hierarchies.

Biology of Memory

July 21–August 3

INSTRUCTORS **C. Alberini,** Mt. Sinai School of Medicine, New York
K. Martin, University of California, Los Angeles
J. Raymond, Stanford University, California

ASSISTANTS **K. Ramamoorthi,** Massachusetts Institute of Technology
J. Rinaldi, Stanford University, California

This lecture course provided an introduction to cellular, molecular, and systems approaches to learning and memory. It was suited for graduate students and postdoctoral fellows in molecular biology, neurobiology, and psychology, as well as research workers who are interested in an introduction to this field. The course covered topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of modern behavioral studies of learning and memory, the cell and molecular biology of neuronal plasticity, cellular and molecular mechanisms of simple forms of learning and memory, and systems approaches to learning in vertebrates and humans. Lectures were complemented by exercises in which students worked in small groups with lecturers to discuss topical issues in learning and memory, evaluated recent studies, and identified and formulated 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.

PARTICIPANTS

Atucha Trevino, E., B.S., University Medical Center, Groningen, The Netherlands
Carcea, I., B.S., New York University, New York
Gerstner, J., Ph.D., University of Pennsylvania, Philadelphia
Groves-Chapman, J., B.S., University of Georgia, Athens
Karlsson, T., B.S., Karolinska Institute, Stockholm, Sweden
McKinney, R., B.S., Massachusetts Institute of Technology, Cambridge
Mitakidis, N., B.S., University of Oxford, United Kingdom
Motley, S., B.S., Brigham Young University, Provo, Utah
Mukherjee, S., Ph.D., Florida International University, Miami
Pancrazi, L., B.S., Scuola Normale Superiore, Pisa, Italy
Resnik, J., B.S., Weizmann Institute of Science, Rehovot, Israel
Rueckemann, J., B.S., Boston University, Massachusetts
Salz, D., B.S., Boston University, Massachusetts
Solyst, J., B.S., Emory University, Atlanta, Georgia
Stern, S., B.S., Mt. Sinai School of Medicine, New York
Tiwari, S., B.S., Institute of Psychiatry, King's College London, London, United Kingdom
Venkataraman, A., B.S., Texas A&M University, College Station
Winter, O., B.S., Stanford University, Menlo Park, California

SEMINARS

Alberini, C., Mt. Sinai School of Medicine, New York: Memory consolidation and reconsolidation.
Byrne, J., University of Texas, Houston Medical School: Cellular and molecular mechanisms of simple forms of learning.
Eichenbaum, H., Boston University, Massachusetts: Toward a neurobiology of declarative memory.
Froemke, R., New York University School of Medicine, New York: Long-term cortical synaptic plasticity.
Goldman, M., University of California, Davis: Computational models of short-term memory.
Holland, P., Johns Hopkins University, Baltimore, Maryland: Learning theory.
Klann, E., New York University, New York: Translational control in memory and brain disorders.
Martin, K., University of California, Los Angeles: Signaling between synapse and nucleus during long-lasting synaptic plasticity.
Raymond, J., Stanford University, California: Cerebellum-dependent learning.
Squire, L., University of California, San Diego/Veterans Affairs Medical Center, San Diego: The neuroscience of memory in historical perspective: 1880–2005. Memory systems of the mammalian brain.
Suzuki, W., New York University, New York: Associative learning and temporal order memory signals in the monkey medical temporal lobe.
Suzuki, W., New York University, New York and Jordan, G., Handel Group, New York, New York: Leadership seminar.
Silva, A., University of California, Los Angeles: Molecular and cellular mechanisms of learning and memory.
Waddell, S., University of Massachusetts Medical School, Worcester: Memory in *Drosophila*.

Eukaryotic Gene Expression

July 26–August 15

INSTRUCTORS
J. Espinosa, HHMI/University of Colorado, Boulder
W. Lee Kraus, Cornell University, Ithaca, New York
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri
D. Taatjes, University of Colorado, Boulder

ASSISTANTS
S. Gadad, University of Texas Southwestern Medical Center, Dallas
M. Galbraith, University of Colorado, Boulder
S.-C. Lin, University of Colorado, Boulder
N. Mohaghegh, Stowers Institute for Medical Research, Kansas City, Missouri
M. Sun, 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 on both in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. Characterizations of the DNA-binding properties of site-specific transcription factors were carried out using electrophoretic mobility shift and DNase I footprinting assays. In addition, students learned techniques for the assembly and analysis of chromatin in vitro, including transcription assays, chromatin footprinting, and chromatin remodeling assays.

During the past few years, the gene regulation field has developed in vivo approaches to study gene regulation. Students were exposed to the chromatin immunoprecipitation technique. They also used RNA interference (RNAi) for specific knock-down experiments in mammalian cells. In

addition, determining cellular gene expression profiles has been accelerated tremendously by DNA microarray technology. Students received hands-on training in performing and interpreting results from DNA microarrays.

Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Lectures by the instructors covered the current status of the gene expression field, theoretical aspects of the methodology, and broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes. Guest lecturers discussed contemporary problems in eukaryotic gene regulation and technical approaches to their solution.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Andino, B., B.A., Mt. Sinai School of Medicine, New York
Ask, K., M.S., University of Copenhagen, Denmark
Beira, J., M.Sc., University College London/National Institute Medical Research London, United Kingdom
Benko, S., Parm.D., Mt. Sinai School of Medicine, New York
Bowman, E., B.S., Emory University, Atlanta, Georgia
Chen, M.-W., B.S., Dana-Farber Cancer Institute, Boston, Massachusetts
Fadul, J., B.S., Huntsman Cancer Institute, Salt Lake City, Utah
Gu, B., B.S., CNRS UMR 8197–INSERM U1024-ENS, Paris, France
McPhee, C., Ph.D., Harvard University, Cambridge, Massachusetts
Moehle, E., B.A., University of California, San Francisco
Nguyen, V., Ph.D., Harvard University, Cambridge, Massachusetts
Perez-Perri, J., M.S., Instituto Leloir, Ciudad de Buenos Aires, Argentina
Price, M., B.A., H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida
Waldholm, J., M.S., Stockholm University, Sweden
Wat, M., B.S., Baylor College of Medicine, Houston, Texas
Woznicki, J., M.Sc., University College Cork, Ireland

SEMINARS

Berger, S., University of Pennsylvania, Philadelphia: The complex language of histone posttranslational modification in genomic regulation.
Conaway, J., Stowers Institute for Medical Research, Kansas City, Missouri: Mediator.
Espinosa, J., HHMI/University of Colorado at Boulder, Boulder: Research presentation.
Garrett, A., Stowers Institute for Medical Research, Kansas City, Missouri: RNA-Req methods.
Gingeras, T., Cold Spring Harbor Laboratory: Lessons learned from complex genomes.
Jones, K., The Salk Institute for Biological Studies, La Jolla, California: A role for transcription elongation factors in gene-specific splicing.
Kingston, R., Massachusetts General Hospital/Harvard Medical School, Boston: Nucleosome position and compaction as mechanisms of epigenetic regulation.
Kraus, L., University of Texas Southwestern Medical Center, Dallas: Research presentation.
Levine, M., University of California, Berkeley: Mechanisms of transcriptional precision in the *Drosophila* embryo.
Lis, J., Cornell University, Ithaca, New York: Dynamic interplay of transcription factors, RNA polymerase II, and chromatin.
Martienssen, R., Cold Spring Harbor Laboratory: RNAi, heterochromatin, and the clash of the polymerases.
Meyer, B., HHMI/University of California, Berkeley: Chromosome-wide mechanisms of gene regulation.
Mostoslavsky, R., The Massachusetts General Hospital, Boston: The histone deacetylase SIRT6 is a master regulator of glucose homeostasis.
Naar, A., Harvard Medical School, Charlestown, Massachusetts: Gene regulatory mechanisms governing cholesterol and lipid metabolism.
Pugh, F., Penn State University, University Park: Genome-wide mapping of chromatin and the transcription machinery at ultra-high resolution.
Shiekhattar, R., The Wistar Institute, Philadelphia, Pennsylvania: The Yin and Yang of noncoding RNAs.
Shilatifard, A., Stowers Institute for Medical Research, Kansas City, Missouri: Research presentation.
Struhl, K., Harvard Medical School, Boston, Massachusetts: Transcription factors, not chromatin, is primarily responsible for epigenetic inheritance.
Taatjes, D., University of Colorado, Boulder: Research presentation.
Tyler, J., University of Texas/MD Anderson Cancer Center, Houston: Chromatin assembly and disassembly.
Wang, Y., University of Texas Southwestern Medical Center, Dallas: Transcriptional regulation of skeletal homeostasis.
Whetstine, J., MCG Cancer Center and Harvard Medical School, Charlestown, Massachusetts: A glimpse into histone demethylase function: Novel roles in cell cycle and behavior.
Yamamoto, K., University of California, San Francisco: Combinatorial transcriptional regulation: Determinants of specificity.
Young, R., Whitehead Institute/Massachusetts Institute of Technology, Cambridge: Transcriptional control of embryonic stem cells.

Imaging Structure and Function in the Nervous System

July 26–August 15

INSTRUCTORS
D. DiGregorio, Université Paris 5, France
S. Thompson, University of Maryland School of Medicine, Baltimore
J. Waters, Northwestern University, Chicago, Illinois

ASSISTANTS
L. Cathala, Universite Pierre et Marie Curie
S. Cerceo, University of Maryland, Baltimore
A. Nimmerjahn, Salk Institute, La Jolla, California
A. Scimemi, National Institutes of Health, Bethesda, Maryland
A. Shih, University of California, San Diego, La Jolla

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes presented expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to use emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy as well as use of different types of electronic cameras, laser scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multiphoton laser-scanning microscopy and to newly available biological fluorophores, especially green fluorescent protein (GFP) and its variants. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells.

Applicants had a strong background in the neurosciences or in cell biology. In their personal statements, applicants specify (1) their experience with optical techniques, (2) how they apply optical methods in their current projects, (3) the microscope systems available to them, and (4) long-term goals in learning more about optical methods.

This course was supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders & Stroke, Howard Hughes Medical Institute, and Coherent, Inc.

PARTICIPANTS

Cho, S., Ph.D., Oregon Health & Science University, Portland, Oregon
Del Marmol, J., M.S., The Rockefeller University, New York
Huang, B., B.A., University of California, Los Angeles
Ikeda, K., Ph.D., Australian National University, Canberra, Australia
Iremonger, K., Ph.D., University of Otago, Dunedin, New Zealand
Lee, K.H.g, Ph.D., University of California, Los Angeles
Matthews, E., B.S., University Clinic Bonn, Germany
Paradiso, K., Ph.D., Rutgers University, Piscataway, New Jersey
Rompani, S., B.S., Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland
Rungta, R., B.S., University of British Columbia, Vancouver, Canada
Watkins, P., Ph.D., University of Maryland, College Park
Weisz, C., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland

SEMINARS

Albeanu, F., Cold Spring Harbor Laboratory: Synaptofluorins and vesicle imaging.
Blanpied, T., University of Maryland School of Medicine, Baltimore: PALM/STORM.
Bruchez, M., Carnegie Mellon University, Pittsburgh, Pennsylvania: New and future indicators.
Deisseroth, K., Stanford University, California: Optogenetics.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Extended 2P imaging and block-face EM.
DiGregorio, D., Universite Paris 5, Paris, France: SLMs/holography.
Dittman, J., Weill Cornell Medical College, New York: Photoactivatable XFPs.
Eggeling, C., Max-Planck Institute for Biophysical Chemistry, Gottingen, Germany: STED and FCS.
Engert, F., Harvard University, Cambridge, Massachusetts: Shot noise. Image J and Fiji.
George, N., Olympus America, Inc., Center Valley, Pennsylvania: BX51 optics.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Germany: XFPs.
Huebener, M., Max-Planck Institute of Neurobiology, Martinsried, Germany: Intrinsic imaging.
Kilborn, K., Intelligent Imaging Innovations, Inc., Santa Monica, California: Deconvolution.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Basic microscopy.
Moomaw, L. (Butch) and Pritchard, K., Hamamastu Photonic Systems, Spring Branch, Texas: Cameras and PMTs. 2P microscopy.
Nimmerjahn, A., Salk Institute, La Jolla, California: Organic calcium indicators: bulk loading.
Petreka, D., HHMI/Columbia University, New York: VSDs.
Shih, A., University of California, San Diego, La Jolla: Imaging blood flow.
Szulczewski, M., Prairie Technology, Middleton, Wisconsin: Confocal microscopy.
Thompson, S., University of Maryland School of Medicine, Baltimore: Flash photolysis.
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.
Tyler, W., Arizona State University, Tempe: Ultrasound imaging and stimulation.
Vogel, S., NIAAA/DICBR, National Institutes of Health, Bethesda, Maryland: FRET and FLIM.
Waters, J., Northwestern University, Chicago, Illinois: Week One Recap. Organic calcium indicators. Home-brew microscope including laser safety.
Weninger, K., North Carolina State University, Raleigh: Single-molecule FRET.
Wilbrecht, L., University of California, Emeryville: GFP applications, spine morphology.
Witkowsi, J., Banbury Center, Cold Spring Harbor Laboratory: Ethics.

Yeast Genetics and Genomics

July 26–August 15

INSTRUCTORS
S. Jaspersen, Stowers Institute for Medical Research, Kansas City, Missouri
J. Smith, University of Virginia Health System, Charlottesville
J. Strathern, National Cancer Institute, Frederick, Maryland

ASSISTANTS
J. Avena, University of Colorado, Boulder
J. Johnson, University of Virginia Health Systems, Charlottesville
C. Smoyer, Stowers Institute, Kansas City, Missouri

This was a modern, state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches were emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques, including various types of yeast transformation, gene replacement with plasmids and 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. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, 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

Burke, M., Ph.D., University of California, Irvine
Diaz-Mejia, J., Ph.D., University of Toronto, Canada
Goyal, S., Ph.D., Princeton University, New Jersey
Herrera, C., Ph.D., MRC Imperial College, London, United Kingdom
Jaeger, P., Ph.D., University of California, San Diego, La Jolla
Kilfoil, M., Ph.D., University of Massachusetts, Amherst
Kokina, A., M.Sc., University of Latvia, Riga, Latvia
Larkin, A., Ph.D., Harvard Medical School, Boston, Massachusetts
Malmgren Hill, S., M.S., Gothenburg University, Goteborg, Sweden
Miermont, A., M.S., Laboratoire Matieres et Systems Complexes, Paris, France
Mikheyev, A., Ph.D., Okinawa Institute of Science and Technology, Kunigami-gun, Japan
Nazeer, F., Ph.D., Tufts University School of Medicine, Boston, Massachusetts
Pena, M., Ph.D., University of North Carolina, Chapel Hill
Ranjan, N., Ph.D., ETH Zurich, Switzerland
Reichelt, P., B.Sc, Max-Planck Institute of Biochemistry, Martinsried/Planegg, Germany
Seeber, A., BSc., Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

SEMINARS

Botstein, D., Princeton University, New Jersey: Experimental evolution studies with yeast. Coordination of growth rate, stress response, and metabolic activity in yeast.
Burke, D., University of Virginia, Charlottesville: Mitotic regulation by two checkpoints in yeast.
Costanzo, M., Saccharomyces Genome Database. Stanford, California: Harnessing the awesome power of yeast with the *Saccharomyces* genome database.
Errede, B., University of North Carolina, Chapel Hill: Kinase control of yeast cell fate.
Gartenberg, M., Robert Wood Johnson Medical School, Piscataway, New Jersey: Sir2 ties transcriptional silencing to sister chromatid cohesion.
Gottschling, D., Fred Hutchinson Cancer Research Center, Seattle, Washington: Organelle deterioration with age: The limits of an interconnected system.
Hartman IV, J., University of Alabama, Birmingham: Measuring yeast gene interactions: Technology development and application to human disease.
Johnson, M., Washington University, St. Louis, Missouri: Feasting, fasting, and fermenting: Glucose sensing and signaling by yeasts.
Rine, J., University of Berkeley, California: Yeast models of human disease.
Rothstein, R., Columbia University, New York: Yeast combination.
Weiss, E., Northwestern University, Evanston, Illinois: Defining information flow in signaling systems: Or, the power of letter words.
Wickner, R., National Institutes of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland: Cytoplasmic inheritance.

Statistical Analysis for Genomic Data

August 2–15

INSTRUCTORS **N. Altman,** Penn State University, University Park
H. Bussemaker, Columbia University, New York
V. Carey, Harvard University, Boston, Massachusetts
M. Reimers, Virginia Commonwealth University, Richmond

ASSISTANT **T. Guennel,** Virginia Commonwealth University, Richmond

High-throughput genomics assays have become pervasive in modern biological research. To properly interpret these data, experimental and computational biologists need to have a firm grasp of statistical methodology. This course was designed to build competence in quantitative methods for the analysis of high-throughput molecular biology data.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Arboleda, V., B.A., University of California, Los Angeles
Bunyavanich, S., M.D., Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts
Busse, D., Dipl., Max-Delbruck Centre for Molecular Medicine, Berlin, Germany
Cheng, W.-C., Ph.D., University of Maryland School of Medicine, Baltimore
Gabel, H., Ph.D., Harvard Medical School, Boston, Massachusetts
Goranskaya, D., M.S., Karolinska Institutet, Stockholm, Sweden
Gregg, C., B.S., Harvard University, Cambridge, Massachusetts
Majithia, A., M.D., Massachusetts General Hospital, Boston
Malik, A., B.S., Harvard Medical School, Boston, Massachusetts
Meyer-Schaller, N., Ph.D., University of Basel, Switzerland
Pascuzzi, P., Ph.D., North Carolina State University, Raleigh
Peterson, K., Ph.D., Harvard University, Cambridge, Massachusetts
Simam, J., B.A., KEMRI/Wellcome Trust Research Programme, Kilifi, Kenya
Tripathi, V., Ph.D., The University of the West Indies, St. Augustine, Trinidad and Tobago

SEMINARS

Altman, N., Penn State University, University Park and Huber, W., EMBL, Heidelberg, Germany: RNA-Seq data analysis. Introduction to course. Experimental design. Differential expression.

Altman, N., Penn State University, University Park and Reimers, M., Virginia Commonwealth University School of Medicine, Richmond: Two-way tables, basic clustering.

Bussemaker, H., Columbia University, New York: Linear models II. Representations of DNA-binding specificity. GO scoring. Motif discovery. Linear models I.

Carey, V., Harvard University, Boston, Massachusetts: eQTL mapping using R/bioconductor.

Culhane, A., Dana Farber Cancer Institute/Harvard, Cambridge, Massachusetts: Reproducible research and cancer.

Davis, S., National Institutes of Health, Bethesda, Maryland: Integrative epigenomics I. Integrative epigenomics II.

Elemento, O., Weill Cornell Medical College, New York: Basics of high-throughput sequencing 1. ChIP-Seq analysis. Three-dimensional chromatin structure revealed by high-C-Seq.

Futcher, B., Stony Brook University, New York: Lessons learned from a successful microarray experiment.

Hansen, K., Johns Hopkins University, Baltimore, Maryland: DNA methylation at high resolution.

Horvath, S., University of California, Los Angeles: Classification. Coexpression networks.

Hughes, T., University of Toronto, Canada: The landscape of sequence speciality for DNA-binding proteins.

Lieb, J., University of North Carolina, Chapel Hill: Research seminar.

Lucito, R., Cold Spring Harbor Laboratory: Methylation and copy-number information identify tumor suppressors.

Reimers, M., Virginia Commonwealth University School of Medicine, Richmond: Precourse for students new to R workshop. Microarrays preprocessing. Basics of bioconductor infrastructure.

Storey, J., Princeton University, New Jersey: Controlling systematic cryptic variation in EQTL studies.

Cellular Biology of Addiction

August 9–15

INSTRUCTORS **A. Bonci,** National Institute on Drug Abuse, Baltimore, Maryland
C. Evans, University of California, Los Angeles
M. Von Zastrow, University of California, San Francisco

ASSISTANTS **A. Taylor,** University of California, Los Angeles
P. Dhara, National Institute on Drug Abuse, Baltimore, Maryland

TECH **M.M. Sanchez,** Pompeu Fabra University, Spain

Drug addiction is the most costly neuropsychiatric disorder faced by our nation. Acute and repeated exposure to drugs produces neuroadaptation and long-term memory of the experience, but the cellular and molecular processes involved are only partially understood. The primary objective of this workshop was to provide an intense dialog on 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 neuroadaptive 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 re-

sponse; tolerance and adaptation at the cellular level; and approaches to exploiting the daunting volume generated by neuroinformatics. This workshop provided an integrated view of current and novel research on neuroadaptive responses to addiction, fostered discussion on collaboration and integration, and provided critical information needed to construct a model of addiction as a disease and identify 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 about the neurobiological consequences of drug use and addiction. The workshop was designed to generate interest in this level of analysis, open conduits for collaborations, and present novel routes to investigating the neurobiology of addictive drugs.

PARTICIPANTS

Akins, C., Ph.D., University of Kentucky, Lexington
Bray, J., Ph.D., The Scripps Research Institute, La Jolla, California
Cahill, C., Ph.D., Queen's University, Kingston, Canada
Calu, D., Ph.D., National Institute on Drug Abuse, Baltimore, Maryland
Dias, C., B.S., Mt. Sinai School of Medicine, New York
Giardino, W., B.S., Oregon Health and Science University, Portland
Gillaman, A., Ph.D., University of Pittsburgh Medical Center, Pennsylvania
Gipson, C., B.S., Medical University of South Carolina, Charleston
Guris, D., M.D., University of Chicago Medical Center, Illinois
Mahajan, S., B.S., State University of New York, Buffalo
Marchant, N., B.S., National Institute on Drug Abuse, Baltimore, Maryland
Michaelides, M., Ph.D., Mt. Sinai School of Medicine, New York
Moeller, S., Ph.D., Brookhaven National Laboratory, Upton, New York
Munoz, M., B.S., Salk Institute, La Jolla, California
Nordenankar, K., Ph.D., Uppsala University, Uppsala, Sweden
Shinday, N., B.S., New England Primate Research Center, Southborough, Massachusetts
Tacelosky, D., B.S., Penn State College of Medicine, Hershey
Trigo, J., Ph.D., Pompeu Fabra University, Barcelona, Spain
Wu, J., Ph.D., Northwestern University, Chicago, Illinois
Yang, K., Ph.D., Baylor College of Medicine, Houston, Texas

SEMINARS

Chavkin, C., University of Washington, Seattle: Kappa opioid signaling mechanisms control addiction risk.
Edwards, R., University of California, San Francisco: The transport of neurotransmitters into synaptic vesicles.
Evans, C., University of California, Los Angeles and M. Von Zastrow, University of California, San Francisco: Course overview. Course conclusions/general discussion. Insights to drug action from the perspective of receptor cell biology.
Goldman, D., NIAAA/LNG/National Institutes of Health, Baltimore, Maryland: From genes to addition: Genome searches for the functional variation underlying heritability.
Heberlein, U., University of California, San Francisco: Genetic model organisms in addiction research: Focus on invertebrates.
Kalivas, P., Medical University of South Carolina, Charleston: Searching for the holy grail of addiction.
Kenny, P., The Scripps Research Institute, San Diego, California: Noncoding RNAs and addiction.
Kieffer, B., Institut Genetique et de Biologie Moleculaire et Cellulaire, France: Genetic mouse models for opioid receptor function and regulation.
Kreek, M.J., The Rockefeller University, New York: Endorphins, stress responsivity, gene polymorphisms, and specific addictions: Bidirectional translational research.
Mackie, K., Indiana University, Bloomington: Cannabinoids.
Nairn, A., Yale University School of Medicine, New Haven, Connecticut: Beyond the dopamine receptor: Signal transduction mechanisms and psychostimulant action.
Nestler, E., Mount Sinai School of Medicine, New York: Transcriptional and epigenetic mechanisms of addiction.
O'Brien, C., University of Pennsylvania, Philadelphia: Translating basic addiction into new treatments.
Palmiter, R., HHMI/University of Washington, Seattle: Dompamine and glutamate signaling in the basal ganglia.
Picciotto, M., Yale University, New Haven, Connecticut: Molecular basis of nicotine addiction.
Pollock, J., NIDA/U.S. Department of Health and Human Services/National Institutes of Health, Baltimore, Maryland: An overview of the genetics program at NIDA.
Schoenbaum, G., University of Maryland, Baltimore: The role of the orbitofrontal cortex in addiction.
Skolnick, P., NIDA/National Institutes of Health, Baltimore, Maryland: Developing medications to treat SUDs: Why haven't we been more successful?
Volkow, N., NIDA/National Institutes of Health, Baltimore, Maryland: The addicted human brain.
Wightman, R.M., University of North Carolina, Chapel Hill: Dopamine neurotransmission during reward-based behaviors.
Witten, I., Stanford University, California: Optogenetic dissection of reward circuitry.

Programming for Biology

October 17–November 1

INSTRUCTORS **S. Prochnick,** DOE–Joint Genome Institute/University of California, Berkeley
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware

CO-INSTRUCTOR **S. Robb,** University of California, Riverside

ASSISTANTS **J.-M. Chia,** Cold Spring Harbor Laboratory
E. Lee, Lawrence Berkeley National Laboratory, California
D. Messina, Stockholm University, Stockholm, Sweden
E. Ross, HHMI/Stowers Institute for Medical Research, Kansas City, Missouri
S. Rynearson, University of Utah, Salt Lake City
A. Wolf, Harvard University, Boston, Massachusetts

A computer is already an indispensable tool for database searches, but the use of web-based tools alone is not enough for today's biologist who needs to access and work with data from myriad sources in disparate formats. This need will become ever more important as new technologies increase 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 learned 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 posed problems using their own data and worked with one another and the faculty to solve them. Final projects formed the basis of publications as well as public biological websites (see, e.g., http://bio.perl.org/wiki/Deobfuscator).

The 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 are able to study 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

Cooper, L., Ph.D., Oregon State University, Corvallis
Bacanamwo, M., Ph.D., Morehouse School of Medicine, Atlanta, Georgia
Buchanan, C., B.S., Vanderbilt University, Nashville, Tennessee
Ciarlariello, D., Ph.D., The Ohio State University, Columbus
Danielsson, F., M.Sc., Royal Institute of Technology, Solna, Sweden
Dutil, J., Ph.D., Ponce School of Medicine, Ponce, Pennsylvania
Hetrick, K., M.S., Johns Hopkins University, Baltimore, Maryland
Jetty, S., Ph.D., The University of Arizona, Tucson
Joseph, V., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Kocher, S., B.S., Harvard University, Cambridge, Massachusetts
Marca, L.R.T., M.Sc., Leipzig University, Germany
Pasternak, J., Ph.D., University of Alberta, Edmonton, Canada
Plouviez, S., Ph.D., Duke University Marine Laboratory, Beaufort, North Carolina
Smyth, D., Ph.D., New York University, New York
Thatcher, S., Ph.D., University of Delaware, Newark
Triant, D., Ph.D., University of Virginia, Charlottesville
Tschopp, P., Ph.D., Harvard Medical School, Boston, Massachusetts
Wright, H., Ph.D., University of Wright, Liverpool, United Kingdom
Yue Cui, J., Ph.D., University of Kansas Medical Center, Kansas City
Zhang, B., Ph.D., Cold Spring Harbor Laboratory
Zhang, Z., Ph.D., University of Massachusetts Medical School, Worcester

SEMINARS

Cain, S., Ontario Institute for Cancer Research, Medina, Ohio: Grows.
Chia, J.-M., Cold Spring Harbor Laboratory: Mysql/db/dbi.
Hide, W., Harvard University, Boston, Massachusetts: NGS1.
Pearson, W., University of Virginia, Charlottesville: Blast I. Blast II.
Prochnik, S., DOE-Joint Genome Institute, Walnut Creek, California: Perl piplines.
Robb, S., University of California, Riverside: Bioperl.
Tisdall, J., Dupont Experimental Station, Wilmington, Delaware: Scientific computing.

X-Ray Methods in Structural Biology

October 17–November 1

INSTRUCTORS **W. Furey,** V.A. Medical Center/University of 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 **L. Koharudin,** University of Pittsburgh School of Medicine, 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 were new to macromolecular crystallography. Topics covered included basic diffraction theory, crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, molecular graphics, structure refinement, structure validation, coordinate deposition, and structure presentation. Participants learned through extensive hands-on experiments. One or more proteins were crystallized and the structure(s) determined by several methods, in parallel with lectures on the theory and informal discussions behind the techniques. Applicants were familiar with the creation and editing of simple text files on Linux workstations using a screen-based editor (either vi or emacs).

This course was supported with funds provided by the National Cancer Institute and the Howard Hughes Medical Institute.

PARTICIPANTS

Alam, S., Ph.D., University of Utah, Salt Lake City
Arai, H., B.S., University of California, Irvine
Bojja, R.S., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania
Burkinshaw, B., B.S., University of British Columbia, Vancouver, Canada
Charlton, L., Ph.D., University of Pittsburgh, Pennsylvania
Gore, S., Ph.D., EMBL-EBI, Cambridge, United Kingdom
Hevener, K., Ph.D., University of Tennessee, Chicago, Illinois
Inada, H., Ph.D., Harvard University, Cambridge, Massachusetts
Jeffers, S., Ph.D., Institut Pasteur, Paris, France
Lee, C.-H., M.S., Oregon Health & Science University, Portland
Masoudi, A., B.S., Duke University School of Medicine, Durham, North Carolina
Neher, S., Ph.D., University of North Carolina, Chapel Hill
Nylander, A., B.S., Umea University, Umea, Sweden
Sevryugina, Y., Ph.D., International Institute of Nano and Molecular Medicine, Columbia, Missouri
Stampfer, S., B.A., Tufts University, Boston, Massachusetts
Wagner, J., B.S., University of Kentucky, Lexington

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, California: Structure refinement.
Cohen, A., Stanford Linear Accelerator Center, California: Remote synchrotron data collection.
Emsley, P., University of Glasgow, Oxford, United Kingdom: Model-building tools in coot.
Furey, W., V.A. Medical Center/University of Pittsburgh, Pennsylvania: Anomalous data collection consideration. Patterson group therapy. Solvent flattening/phase combination. Noncrystallographic symmetry averaging. MAD phasing: A classical approach. Solving structures with BnP.
Gilliland, G., Centorcor Inc., Radnor, Pennsylvania: Welcome and introduction. Course overview: The structure determination of biological macromolecules. Maximizing crystallization success through seeding.
Holton, J., University of California, San Francisco: Tips and tricks for improving diffraction.
Kleywegt, G., University of Uppsala, Sweden: Just because it's in Nature, doesn't mean it's true… (macromolecular structure validation).
McPherson, A., University of California, Irvine: Crystallization of macromolecules I. 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. Crystal growth mechanisms.
Pflugrath, J., Rigaku Americas Corporation, The Woodlands, Texas: Data collection: Design and setup. Cryocrystallography. HKL2000 tutorial. Scaling and merging synchrotron data.
Ready, R., University of Cambridge, United Kingdom: Using SAD data in phaser. Molecular replacement: New structures from old.
Richardson, J., Duke University Medical Center, Durham, North Carolina: Structure presentation.
Richardson, J. and Richardson, D., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using MolProbity.
Sweet, R., Brookhaven National Laboratory, Upton, New York: A slightly different view of fundamental crystallography. X-ray sources and optics.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Automated structure solution and model-building.
Tronrud, D., Oregon State University, Corvalis: Macromolecular refinement I. Macromolecular refinement II. Difference electron density maps.
Westbrook, J., Rutgers University, Piscataway, New Jersey: Automating PDB deposition.
Yeh, J., University of Pittsburgh, School of Medicine, Pennsylvania: Approaches for membrane protein crystallization.

Quantitative Imaging: From Cells to Molecules

October 24–November 6

INSTRUCTORS
B. Huang, University of California, San Francisco
M. Krummel, University of California, San Francisco
A. North, The Rockefeller University, New York
S. Reck-Peterson, Harvard Medical School, Boston, Massachusetts
J. Waters, Harvard Medical School, Boston, Massachusetts
T. Wittmann, University of California, San Francisco

ASSISTANTS
D. Kamiyama, University of California, San Francisco
H. Pemble, University of California, San Francisco
L. Piedmont, Nikon Imaging Center at Harvard Medical School, Boston, Massachusetts
W. Qiu, Harvard Medical School, Boston, Massachusetts
J. Rosenberg, Harvard Medical School, Boston, Massachusetts
S. Stehbens, University of California, San Francisco
K. Thomas, The Rockefeller University, New York
J. Wong, University of California, San Francisco

This course focused on advanced quantitative fluorescence microscopy techniques used for imaging a range of biological specimens, from cells to single molecules. The course was designed for cell and molecular biologists with little or no microscopy experience who wish to begin utilizing microscopy in their own research. Students gained a theoretical understanding of, and hands-on experience with, state-of-the-art equipment used in quantitative fluorescence microscopy, including laser-scanning and spinning-disk confocal microscopy, deconvolution methods, total internal fluorescence microscopy (TIRF), superresolution methods (structured illumination, STORM, and PALM), and digital image processing and analysis. Students learned how to design and implement a wide range of imaging experiments using these techniques. Students used the techniques to address specific

quantitative questions and then discussed the results as a group, learning to troubleshoot the common problems that occur in the course of a quantitative imaging experiment. Among the lectures presented were microscopy basics, CCD cameras, confocal microscopy, multiphoton microscopy, deconvolution, TIRF, single-molecule imaging, imaging ratio-metric "biosensors" (including FRET), and superresolution techniques. Students also learned specimen preparation for microscopy, including fixation and immunofluorescence in tissue culture cells, choosing fluorescent proteins, and working with live samples requiring environmental control.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Auld, D., Ph.D., Novartis Institutes for Biomedical Research, Cambridge, Massachusetts
Beck, J., Dipl., ETH Zurich, Switzerland
Didier, R., B.S., Florida State University College of Medicine, Tallahassee
Gonzalez E., M., B.A., Genentech, South San Francisco, California
Hoepker, A., Ph.D., Stanford Medical School & HHMI, California
Hombach-Klonis, S., Ph.D., University of Manitoba, Winnipeg, Canada
Johnson, J., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Lambert, T., Ph.D., Washington State University, Pullman
McClelland, A., Ph.D., Harvard University, Cambridge, Massachusetts
Palacio, S., B.S., Institute of Neurobiology, MSC, University of Puerto Rico, San Juan
Park, H.-O., Ph.D., Ohio State University, Columbus
Paul, A., Ph.D., Cold Spring Harbor Laboratory
Rogers,K., Ph.D., Harvard University, Cambridge, Massachusetts
Sims, C., Ph.D., Harvard University, Boston, Massachusetts
Verver, D., M.Sc., Academic Medical Center Amsterdam, The Netherlands
Wheeler, T., B.S., University of Rochester, New York

SEMINARS

Goodwin, P., Applied Precision Inc., Issaquah, Washington: Deconvolution.
Huang, B., University of California, San Francisco: Superresolution microscopy: STORM/PALM. Superresolution microscopy: Structured illumination. Molecular complexes under the STORM.
Khodjakov, A., Wadsworth Center, Albany, New York: Mitosis in 3D: Spatial constraints and facilitating mechanisms in spindle assembly.
Krummel, M., University of California, San Francisco: Principles, benefits, and applications of multiphoton microscopy. Visualization of immune responses in peripheral tissues.
North, A., The Rockefeller University, New York: Objective lenses. Fluorescence microscopy. Sample preparation for fluorescence microscopy. Live-cell imaging.
Reck-Peterson, S., Harvard Medical School, Boston, Massachusetts: TIRF and single-molecule imaging. The mechanism and regulation of molecular motors.
Waters, J., Harvard Medical School, Boston, Massachusetts: Microscopy basics. Transmitted light microscopy. Digital cameras. Digital imaging. Ratio imaging and FRET.
Wittmann, T., University of California, San Francisco: Confocal microscopy. How microtubule + TIP proteins shape cell function. Live confocal, FRAP, and photoactivation.

Advanced Sequencing Technologies and Applications

November 8–21

INSTRUCTORS

E. Mardis, Washington University School of Medicine, St. Louis, Missouri
G. Marth, Boston College, Chestnut Hill, Massachusetts
W.R. McCombie, Cold Spring Harbor Laboratory
A. Quinlan, University of Virginia, Charlottesville
M. Zody, Broad Institute, Cambridge, Massachusetts

ASSISTANTS

A. Farrell, Boston College, Chestnut Hill, Massachusetts
M. Griffith, Washington University School of Medicine, St. Louis, Missouri
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
J. Walker, Washington University School of Medicine, St. Louis, Missouri

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, moving 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 were explored including DNA resequencing of human genomic regions (using cancer samples as a test case), de novo DNA sequencing of bacterial genomes, and the use of these technologies in study-

ing 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 meeting was sponsored by Applied Biosystems, Illumina, and 454 Life Sciences.

PARTICIPANTS

Bennett, L., Ph.D., Baylor Research Institute, Dallas, Texas
Cox, L., B.S., New York University, New York
De, S., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
de las Nieves Perigones Borderias, M., Ph.D., Children's Hospital of Philadelphia, Pennsylvania
Hanna, M., M.S., Dana Farber Cancer Institute, Boston, Massachusetts
Jacobsen, M., Ph.D., University of Copenhagen, Frederiksberg C., Denmark
Johansson, I., M.S., Lund University, Sweden
Lindsay, C., Ph.D., University of Pennsylvania, Philadelphia
Maree, H., Ph.D., Agricultural Research Council, Stellenbosch, South Africa
Murtaza, M., M.B., B.S., Cancer Research UK, Cambridge, United Kingdom
Pirooznia, M., Ph.D., Johns Hopkins University, Baltimore, Maryland
Pratap, S., Ph.D., Meharry Medical College, Nashville, Tennessee
Rozenzhak, S., Ph.D., University of California, San Diego, California
Trompouki, E., Ph.D., Children's Hospital Boston, Boston, Massachusetts
Williams, A., B.S., Princeton University, New Jersey
Williams, S., Ph.D., University of Virginia, Charlottesville

SEMINARS

Fiume, M., University of Toronto, Canada: Lecture and tutorial on Savant software package.
Griffith, M., BC Cancer Agency, Vancouver, Canada: RNA-Seq lecture and tutorial.
Hall, I. and Quinlan, A., University of Virginia, Charlottesville: Structural variant detection.
Hannon, G., Cold Spring Harbor Laboratory: Whole-genome methylation.
Hicks, J., Cold Spring Harbor Laboratory: Sequencing from a single human cell.
Mardis, E., Washington University School of Medicine, St. Louis, Missouri: Next-generation cancer genomics.
Nekrutenko, A., Penn State University. University Park: Galaxy ChIP-Seq and metagenomics.
Robinson, J., Broad Institute, Cambridge, Massachusetts: The IGV viewer.
Stoops, K., Oxford Nanopore, United Kingdom, Oxford: Nanopore.
Yandell, M., University of Utah. Salt Lake City: The VAAST software tool. Lecture and tutorial.

C. elegans

November 8–21

INSTRUCTORS
M. Alkema, University of Massachusetts Medical School, Worcester
E. Lambie, LMU—Martinsried, Planegg-Martinsried, Bayern, Germany
E. Miska, University of Cambridge, United Kingdom

ASSISTANTS
D. King, Cold Spring Harbor Laboratory
J. Pirri, University of Massachusetts Medical School, Worcester
S. Rolland, Ludwig Maximilian University, Munich, Germany
A. Sapetschnig, University of Cambridge, United Kingdom

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis and reverse genetic approaches. A major goal was to teach students how to successfully exploit the information generated by the *C. elegans* genome project. The course was suited both for those who have current training in molecular biology and some knowledge of genetics, but 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 them 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

Arczewska, K., Ph.D., The Biotechnology Centre of Oslo, Norway
Bonilla, A., B.S., Dartmouth College, Hanover, New Hampshire
Hernando, G., B.S., National Research Council of Argentina, Bahia Blanca, Argentina
Huelgas Morales, G., Ph.D., Instituto de Fisiologia Cellular, UNAM, Mexico City, Mexico
Ly, K., B.Sc., University of Auckland, New Zealand
Maia, A., B.S., University Medical Center Utrecht, The Netherlands
Martell, J., B.S., Boston College, Chestnut Hill, Massachusetts
Pieczynski, J., Ph.D., University of Alabama, Birmingham
Reymann, A.-C., Ph.D., CEA Grenoble, France
Riehs-Kearnan, N., M.S., University of California, San Francisco
Sarkies, P., M.B., MRC Laboratory for Molecular Biology, Cambridge, United Kingdom
Shibata, Y., Ph.D., Northwestern University, Evanston, Illinois
Stuart, R., Ph.D., Marquette University, Milwaukee, Wisconsin
Vergara, S., Ph.D., University of Massachusetts Medical School, Worcester
Visscher, M., M.S., University of Medical Center Utrecht, The Netherlands
Wook Jung, K., B.S., Stanford University, California

SEMINARS

Alkema, M., University of Massachusetts Medical School, Worcester: The neuroethology of *C. elegans* escape behavior.
Ambros, V., University of Massachusetts Medical School, Worcester: microRNAs and developmental timing in *C. elegans*.
Antebi, A., Baylor College of Medicine, Houston, Texas: The neuroethology of *C. elegans* escape behavior.
Cutter, A., University of Toronto, Ontario, Canada: The genetic basis of evolutionary change in Nematoda.
Greenstein, D., University of Minnesota, Nashville: Signaling for sex in *C. elegans*.
Hall, D., Albert Einstein College of Medicine, Bronx, New York: Introduction on *C. elegans* anatomy. Modern EM methods for nematode.
Jorgensen, E., University of Utah, Salt Lake City: Engineering the genome with the Mos transposon.
Lambie, E., LMU-Martinsried, Planegg-Martinsried, Bayern, Germany: Forward and reverse genetic analyses of TRPM channel function in *C. elegans*.
Miska, E., University of Cambridge, United Kingdom: The complex life of small RNA.
Rockman, M., New York University, New York: Natural genetic variation as a research tool.
Schnabel, R., Institut fuer Genetik, Braunschweig, Germany: A practical approach to multichannel 4D microscopy: Phainothea and pattern formation by cell focusing in the *C. elegans* embryo.
Walhout, M., University of Massachusetts, Worcester: Gene regulatory networks.

Computational and Comparative Genomics

November 9–15

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
L. Stubbs, University of Illinois, Urbana

ASSISTANT **L. Mills,** University of Virginia, Charlottesville

This course presented a comprehensive overview of the theory and practice of computational methods for the identification and characterization of functional elements 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 the following:

- Alignment and analysis of "next-gen" sequencing data, with applications from metagenomic, RNA-Seq, and CHiP-Seq experiments.
- The Galaxy environment for high-throughput analysis.
- Regulatory element and motif identification from conserved signals in aligned and unaligned sequences.
- Integration of genetic and sequence information in biological databases.
- The ENSEMBL genome browser and BioMart.

The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course was designed for biologists seeking advanced training in biological sequence and genome analysis, computational biology core resource directors and staff, and for individuals in other disciplines (e.g., computer science) who wished to survey current research problems in biological sequence analysis. Advanced programming skills were not required.

The primary focus of this course was the theory and practice of algorithms in computational biology, with the goals of using current methods more effectively for biological discovery and developing new algorithms. Students more interested in the practical aspects of software development

are encouraged to apply to the course on Programming for Biology. Students who would like in-depth training in the analysis of next-generation sequencing data (e.g., SNP calling and the detection of structural variants) are urged to apply to the course on Advanced Sequencing Technologies and Applications for next year.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Aitken, K., Ph.D., CSIRO, St. Lucia, Australia
Belleau, P., M.S., CHUL Research Center, Quebec, Canada
Borhan, H., Ph.D., AAFC, Saskatoon, Canada
Borstrom, E., M.S., Science for Life Laboratory, Solna, Sweden
Coykendall, K., Ph.D., USGS-Leetown Science Center, Kearneysville, West Virginia
Creasey, K., Ph.D., Cold Spring Harbor Laboratory
Fu, Q., M.S., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Garg, G., B.Sc., Technical University of Denmark, Kongenss. Lyngby, Denmark
Koroteev, M., Ph.D., Okinawa Institute of Science and Technology, Onnason, Japan
Lu, W., B.S., Indiana University School of Medicine, Indianapolis
Ntziachristos, P., B.Sc., HHMI/New York University School of Medicine, New York
Oza, J., B.S., Robert Wood Johnson Medical School, New Brunswick, New Jersey
Pearl, S., B.S., University of Georgia, Athens
Potdar, A., M.S., CWRU/Cleveland Clinic, Cleveland, Ohio
Rubio Garcia, A., B.Sc., Technical University of Denmark, Denmark
Stafford, G., Ph.D., The Jackson Laboratory, Bar Harbor, Maine
Stevison, L., Ph.D., University of California, San Francisco
Sweigart, A., Ph.D., University of Georgia, Athens
Yang, Y., Ph.D., The University of Texas/MD Anderson Cancer Center, Smithville
Zhou, C., B.Sc., University of Alberta, Canada

SEMINARS

Mackey, A., University of Virginia, Charlottesville: Signaling for sex in *C. elegans*.
Overduin, B., EMBL–European Bioinformatics Institute, Cambridge, United Kingdom: The ENSEMBL database of genomes I. Ensembl/Biomart.
Quellette, F., Ontario Institute for Cancer Research, Toronto, Canada: Databases for computational biology. Pathway databases.
Smith, A., University School of California, Los Angeles: Functional elements II: Epigenomics.
Stubbs, L., University of Illinois, Urbana and Smith, A., University School of California, Los Angeles: Identifying functional elements I.
Stubbs, L., University of Illinois, Urbana: Next-gen biology: Chromatin and transcription factor maps.
Taylor, J., Emory University, Atlanta, Georgia: Galaxy for high-throughput analysis.
White, O., University of Maryland School of Medicine, Baltimore: Introduction to metagenomics.

The Genome Access Course

April 19–20, November 29–30

TRAINERS

G. Howell, The Jackson Laboratory
B. King, Mount Desert Island Biological Laboratories
C. Lambert, Cold Spring Harbor Laboratory

This course is an intensive 2-day introduction to bioinformatics that was held twice in 2011 and trained almost 70 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 19–20

Burns, C., Washington & Jefferson College, Washington, Pensylvania
Cheng, S., Brigham & Women's Hospital, Cambridge, Massachusetts
Ge, H., Peking University, Beijing, China
Hersch, J., Whitehead Institute, Cambridge, Massachusetts
Hultman, K., Northwestern University, Evanston, Illinois
Justiniano, E., University of Puerto Rico, Mayaguez
Mitra, D., Harvard Medical School/Massachusetts General Hospital, Charlestown
Moragoda, L., Henry Ford Community College, Dearborn, Michigan
Podur, J., York University, Toronto, Canada
Robb, E., Brock University, St. Catherine's, Canada
Root, P., Henry Ford Community College, Dearborn, Michigan
Shaikhibra, Z., University Hospital, Faculty of Medicine, Bonn, Germany
Solomon, R., Cold Spring Harbor Laboratory, New York
Stepaniak, J., Henry Ford Community College, Dearborn, Michigan
Tang, J., Metabolix, Cambridge, Massachusetts
Wang, V., Massachusetts General Hospital, Boston, Massachusetts
Webster, K., Brown University, Providence, Rhode Island
Wray, L., Children's Hospital of Philadelphia, Pennsylvania
Wulf, H., Bartlesville, Oklahoma
Wulf, K., Bartlesville, Oklahoma
Xenopoulos, P., Memorial Sloan-Kettering Cancer Center, New York
Yoon, H.J., VA Loma Linda HealthCare, Irvine, California

November 29–30

Agshe, D., Harvard University, Cambridge, Massachusetts
Alvarez, D., Harvard Medical School, Boston, Massachusetts
Bekdash, R., Rutgers University, New Brunswick, New Jersey
Breton, T., University of New Hampshire, Durham
Cai, Y., Cold Spring Harbor Laboratory, New York
Chau, J., University of Connecticut, Manchester
Cong, L., New York Presbyterian Hospital/Weill Medical Center, New York
Congdon, C.B., University of Southern Maine, Portland
Demeure, M., Translational Genomics Research Institute, Scottsdale, Arizona
Everett, L., University of Michigan, Ann Arbor
Fendler, B., Cold Spring Harbor Laboratory, New York
Guha, U., Medical Oncology Branch, NCI, Bethesda, Maryland
Hall, L., Harvard Medical School, NEPRC, Southborough, Massachusetts
Harbour, K., USDA, Maricopa, Arizona
Ho, J., Forage Genetics International, Madison, Wisconsin
Hu, H., SUNY Upstate Medical University, Syracuse, New York
Imumorin, I., Cornell University, Ithaca, New York
Ko, G.P., Seoul National University, South Korea
Kothera, L., Centers for Disease Control and Prevention, Fort Collins, Colorado
Lee, H.-A., Boyce Thompson Institute for Plant Research, Ithaca, New York
Lenz, P., University of Hawaii at Manoa, Honolulu
Maglic, D., Wake Forest University School of Medicine, Winston-Salem, North Carolina
Morgan, J., Harvard Medical School, NEPRC, Southborough, Massachusetts
Ni, E., Regeneron Pharmaceuticals Inc., Tarrytown, New York
Nicolette, P., Columbia University, New York
Slabodnick, M., University of California, San Francisco
Stanek, T., Thomas Jefferson University, Philadelphia, Pennsylvania
Stueve T., Mount Sinai School of Medicine, New York
Tembe, W., TGen, Translational Genomics Institute, Phoenix, Arizona
Tombert, K., University of Michigan, Ann Arbor
Werling, D., University of California, Los Angeles
Zhang, S., University of Texas/MD Anderson Cancer Center, Houston

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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. David Scadden, Massachusetts General Hospital, Harvard University	Niche-induced oncogenesis.	Grisha Enikolopov
Dr. Jeffrey Engelman, Massachusetts General Hospital Cancer Center/Harvard Medical School	Targeted therapies.	Lloyd Trotman
Dr. David Stern, Princeton University	How a complex enhancer contributes to phenotypic robustness and morphological evolution.	Leemor Joshua-Tor
Dr. Douglas Green, St. Jude Children's Hospital	Metabolic reprogramming in activated T lymphocytes.	Richard Sever
February		
Dr. Frederick DeSauvage, Genentech, Inc.	Targeting the hedgehog pathway in cancer: From bench to clinic.	Scott Powers
Dr. Daniel Geschwind, University of California, Los Angeles	Autism: A model for integration of genomics, genetics, and neurobiology in complex neurodevelopmental disorders.	Pavel Osten
Dr. Randall Johnson, Moores Cancer Center, University of California, San Diego Medical Center	The physiology of hypoxic response: The yin and yang of HIF-1 and HIF-2.	Mikala Egeblad
Dr. Gabriele Bergers, University of California, San Francisco	Feedback control of the microenvironment in tumor progression and resistance.	Mikala Egeblad
March		
Dr. Frederick "Fritz" Roth, University of Toronto	Bar-code fusion genetics, entangled mate sequencing, and green monsters.	Ivan Iossifov
Dr. Arnold Kriegstein, University of California, San Francisco	Neural stem and progenitor cells in human cortical development.	Josh Huang
Dr. Nicholas Proudfoot, University of Oxford	Gene punctuation in eukaryotes: Gene loops, R-loops, and noncoding RNA.	Leemor Joshua-Tor
Dr. Christopher Miller, Brandeis University	Puzzles of molecular recognition in a virtual proton pump.	Hiro Furukawa
Dr. Wolf Reik, Babraham Institute, UK	Epigenetic reprogramming in mammalian development.	Rob Martienssen
April		
Dr. Gero Miesenböck, University of Oxford	Lighting up the brain.	Josh Dubnau
Dr. Peter Walter, University of California, San Francisco	The unfolded protein response in health and disease.	Adrian Krainer
Dr. Steven F. Dowdy, University of California School of Medicine, San Diego	The road to therapeutic RNAi: Tackling the siRNA delivery gorilla.	Adrian Krainer
Dr. Kornelia Polyak, Harvard Medical School and Dana Farber Cancer Institute	Diversity in breast cancer: Relevance and therapeutic implications.	Mikala Egeblad
October		
Dr. Robert Full, University of California, Berkeley	Neuromechanical systems biology: Gripping geckos, crashing cockroaches, and galloping ghost crabs.	Katie Liberatore and Matt Camiolo
Dr. James Allison, Memorial Sloan-Kettering Cancer Center	Immune checkpoint blockade in cancer therapy: New insights and opportunities.	Lloyd Trotman

	Title	Host
October (*continued*)		
Dr. Massimo Scanziani, University of California, San Diego	Addressing cortical processing by perturbing the activity of individual layers.	Bo Li
November		
Dr. Douglas Green, St. Jude Children's Research Hospital	ER–mitochondrial interactions in apoptosis: A "greasy" road to ruin.	Richard Sever
Dr. Allan Bradley, The Wellcome Trust Sanger Institute, United Kingdom	Genetics in stem cells: From gene discovery to therapy.	Alea Mills
December		
Dr. Bob Darnell, The Rockefeller University	Maps of the RNA world.	Adrian Krainer

IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor Laboratory's In-House Seminars were initiated to provide a semiformal avenue for communication among the various research groups at the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

January	**Title**
Yuntao "Steve" Mao (Spector Lab)	Direct visualization of the cotranscriptional assembly of a nuclear body by noncoding RNAs.
Bo Li	Excitatory synapse and psychiatric disorders.
Christopher Hammell	Developmental regulation of microRNA activity.
February	
Agustin Chicas (Lowe Lab)	The RB-binding proteins JARID1A/JARID1B link the RB tumor suppressor to epigenetic reprogramming during cellular senescence.
Hiro Furukawa	Structural dissection of NMDA receptor pharmacology.
Darryl Pappin	New proteomics technologies.
March	
Jer-Ming Chia (Ware Lab)	Navigating the maze: Tracing the genetic architecture of traits in a fluid genome.
Thomas Schlach (Joshua-Tor Lab)	Coupling RNAi to heterochromatin: Insights into the structure of the RITS complex.
Adam Kepecs	Decisions, uncertainty, and the brain: Neural circuit mechanisms underlying cognition.
April	
Anne Churchland	Putting the pieces together: How neural systems integrate information across time and sensory modalities.
Hongwu Zheng	Understanding glioma pathogenesis.
Benjamin Boettner (Van Aelst Lab)	Rap1 signaling at a critical junction to cell–cell adhesion.
October	
Navasona Krishnan (Tonks Lab)	Something smells in signal transduction.
Glenn Turner	Reading the mind of a fly: Neural coding in the *Drosophila* mushroom body.
Mickey Atwal	Cancer: A quantitative story of stress, sex, and evolution.
November	
Lloyd Trotman	Principles of tumor suppression: Translating cancer genetics into biology.
Stephen Shea	Neural circuitry for social communication in mice.
Shipra Das (Krainer Lab)	Mechanisms of splicing misregulation in cancer and senescence.
December	
Michael Schatz	Applications of micro-, mega-, and meta-assembly.


BANBURY CENTER

BANBURY CENTER EXECUTIVE DIRECTOR'S REPORT

It was another busy year for the Banbury Center. The Center was used for 34 events with a total of 686 participants. There were 24 science-related meetings, together with six lecture courses run by the Meetings and Courses Program and two Watson School *Topics in Biology* courses. Of the 686 participants, 511 were from the United States (from 35 different states) and 96 from Europe (16 different countries). The truly international character of the Center's program is shown by the fact that participants came from a total of 25 countries worldwide.

Banbury Center fosters and promotes research by providing a venue for meetings other than those dealing with scientific topics, for example, by providing training for young scientists and physicians at the beginnings of their careers. The National Institute of Mental Health held its third "Brain Camp" for young physicians, to introduce them to the latest basic research relevant to their work. The Boehringer Ingelheim Fonds returned with its fellows drawn from Europe and North America for a "Communicating Science" workshop. The fellows are subjected to a rigorous training in writing skills, presentation of graphic information, and preparing and delivering a scientific talk, so that they can excel in these skills as well as their research. For the first time, we held a "Leadership in Bioscience" workshop. David Stewart received funding from the American Express Foundation under its Leadership Program for a short course where participants were to be instructed in things scientists must be able to do—in addition to their research—to succeed. The first course was held in 2011, organized by Carl Cohen, coauthor of *Lab Dynamics: Management Skills for Scientists*, published by Cold Spring Harbor Laboratory Press. The course drew participants from institutes throughout the United States.

We also promote research by hosting meetings where a small number of members of groups or associations come together to discuss policy affecting their work. There were four such examples this year. The National Cancer Institute (NCI) established a network of biobanks to act as clearing houses to collect and supply biopsies, DNA, and cell cultures to researchers. In June, representatives of the NCI biobanks came to Banbury to review the effectiveness and discuss the future of the program. Discussions were guided by Laurence Baker (University of Michigan Cancer Center) and Scott Lowe (CSHL), with input from Harold Varmus, Director of NCI.

We have held several meetings on chronic fatigue syndrome and this year we were delighted to host the Scientific Advisory Board of the Chronic Fatigue and Immune Dysfunction Syndrome (CFIDS) Association. During the past few years, CFS research has received high-profile media attention, for example, over the claim (now discredited) that the XMR virus is a cause of CFS. This has generated both increased interest and unprecedented opportunities for progress. The goals of the meeting were for the Scientific Advisory Board to provide guidance on research strategies to encourage innovative research focused on early detection, objective diagnosis, and effective treatment.

This group also included a meeting of the editorial board of the Federation of Biochemical Societies (*FEBS*) journal. Banbury Center provided a quiet haven for the board to meet with the journal's staff and discuss the performance and future direction of the journal.

Finally, representatives of the Epigenomics of Plants International Consortium (EPIC), funded by the National Science Foundation (NSF), came to Banbury in November. The Consortium was established to coordinate and promote research on deciphering the plant epigenome. Participants discussed intellectual questions, transformative methodologies, and infrastructure needs required to do this, as well as the means to engage funding agencies and the international research community as a whole.

The broad sweep of scientific topics of Banbury Center meetings changes from year to year. One year, it may be neuroscience and psychiatric disorders, and another year, it may be human genetic disorders. This year, the focus was on cancer, in particular on treatments for cancer. The first meeting, *Curing Melanoma and Other Cancers by Targeted Therapies*, was organized by Joseph Schlessinger (Yale University) and Jim Watson. It could not have been held at a more auspicious moment. In the course of the meeting, Jim Allison (Memorial Sloan-Kettering Cancer Center) learned that the Food and Drug Administration (FDA) had approved a monoclonal antibody therapy for metastatic melanoma that had been developed based on his work.

The second cancer meeting was *Metformin and Neoplasia*. Metformin is an antidiabetic drug used to treat type II diabetes. It is the most widely prescribed antidiabetic in the world. Remarkably, epidemiological studies have shown that patients taking metformin have a lower risk of developing cancer. The mechanism(s) underlying this anticancer effect is far from clear and was a focal point of the presentations and discussions at the meeting. Metformin has an added interest in the context of "repurposing" drugs. It is argued that drugs such as metformin, which have been given to hundreds of millions of people and whose safety is established, should be fast-tracked for approval for other uses.

As long ago as 1995, Stan Prusiner organized the first Banbury Center meeting on prions. At that time, there were many controversial issues about the nature, replication, and species specificity of these misfolded proteins. Prion-like particles became immensely important to the population of the United Kingdom in 1990 when patients were found suffering from what was called variant Creutzfeldt–Jakob disease. The cause of the disease was demonstrated to be meat contaminated with neural material from cattle suffering with bovine spongiform encephalopathy. As a result, a large proportion of the United Kingdom population had been exposed, leading to almost 200 deaths. Misfolded proteins are found in other disorders (for example, Alzheimer's disease), and there is recent evidence that Alzheimer's disease amyloidosis can be transmitted to primates. That these disorders might be transmissible has important implications for public health, and the time was clearly right for a critical review of the data on transmission of the amyloidoses, the mechanisms involved, and the implications for human health.

Another area in which Banbury Center has long had an interest is the genetics of psychiatric disorders. In the early 1990s, we held several meetings reporting on the progress of finding genes involved in these disorders using the only tool then available, linkage analysis. A later incarnation of this approach, genome-wide association studies, has found many locations associated with psychiatric disorders, but the significance of these remains unknown. Now, new high-throughput DNA sequencing techniques have made, or are about to make, it possible to sequence the whole exomes and genomes of large numbers of individuals. This will provide opportunities to develop new gene-hunting strategies for complex genetic disorders. Our meeting brought together experts to critically assess current strategies and to outline how genome-scale sequencing can be used most effectively and efficiently.

The Cold Spring Harbor Laboratory Corporate Sponsor Program is a mainstay of the Banbury Center program, providing funding each year for a small number of meetings. We are very grateful to members of the Program for their support and happy when they wish to use Banbury Center for a meeting of their own choice. In 2011, both Astellas-OSI Pharmaceuticals and Sanofi-Aventis came to Banbury.

The Banbury Center could not operate at its high level without the hard work of many people. The Center is especially fortunate to have Janice Tozzo and Susanne Igneri ensuring that the meetings run smoothly, and Basia Polakowski making sure that participants are welcome in Robertson House. Sonny Leute, Fredy Vasquez, and Joseph McCoy look after the grounds, dealing with vast amounts of leaves in the fall and, this year, vast amounts of snow in the winter. Jon Parsons is indefatigable in handling audio-visual requirements, and Connie Brukin enlivens this report with her photographs. Culinary Services feeds our participants and Housekeeping copes admirably with the rapid turnover of guests.

Garage

Renovations, 1976

It is now 35 years since the garage of the Robertsons' estate was converted into the Conference Room, and for the first time in that period, the room is undergoing a complete renovation. It is long overdue. In particular, the original wiring was not designed to take the load imposed by all of the computers that participants bring or the demands of modern projectors and copying machines. We will also upgrade all of the ethernet cabling and generally bring the facility into the 21st century. The building has been emptied and turned over to the electricians, carpenters, and painters—and we look forward to hosting meetings for the next 35 years!

Jan Witkowski
Executive Director

BANBURY CENTER MEETINGS

Leadership in Bioscience Workshop

February 18–21

FUNDED BY **The American Express Foundation**

ARRANGED BY **C. Cohen,** Science Management Associates, Newton, Massachusetts
D. Kennedy, WorkLab, LLC, New York

A scientist running a laboratory is essentially running a business, small to begin with but likely to get larger with time. And so, scientists need to develop skills akin to those needed to run a business: identifying and resolving conflicts, dealing with difficult people, leading effective and productive meetings, and communicating effectively within the laboratory and with the outside world. These skills, if acquired at all, are usually learned haphazardly, after the fact. How much better to learn them systematically and in advance of needing them! So, David Stewart, Executive Director of the Meetings and Courses Program, applied to the American Express Foundation for funding from the Foundation's Leadership Program. He was successful and the "Leadership in Bioscience Workshop" was the result.

SESSION 1: Who We Are

Participants read their 50–100-word "Who I am and what I hope to get from this workshop" aloud to the entire group. They revealed to the group what they would be if they were not scientists.

SESSION 2: Introduction: What Is Leadership and What Makes a Great Scientist/Leader?

Part 1. Small groups proposed attributes of leadership especially in a scientific context, and they discussed examples of effective and ineffective leadership based on their own experience and observations.

Part 2. Small groups reported to the entire group. The goal was to develop attributes of excellent leaders to serve as a reference point for the rest of the conference.

SESSION 3: Difficult Conversations and Interactions

Participants explored the types of situations that scientists find difficult as they transition into leadership positions and received a practical toolkit to use in those situations.

- Learning to listen and to seek out underlying interests
- Fundamental tools for negotiating difficult conversations
- Dealing with difficult people

SESSION 4: Keynote Speaker: Zia Khan, Vice President, Strategy and Evaluation, Rockefeller Foundation, New York

SESSION 5: Case Studies

Attendees were instructed to bring a one-page case study describing a difficult management situation or leadership challenge that they have faced or are facing.

Part 1. In small groups, each attendee read their case aloud. A structured discussion guide was provided to elicit comments, discussion, and suggestions from the group in the context of the work already done in the workshop. Each small group selected one case that best illustrated a key leadership challenge for presentation in summary to the entire group.

Part 2. Each small group made a 1-minute summary of its selected case to the large group, which then chose just one or more cases to discuss in the large group. Large groups' ideas/suggestions/approaches were compared with those of the small group. A discussion of leadership characteristics was tied in from Session 1.

SESSION 6: Group Dynamics and Meetings

- How to run/lead meetings
- How to structure and encourage open discussion, ensuring participation
- How to deal with silence and nonparticipants
- How to recognize and manage impediments to effective group problem solving

SESSION 7: Projecting Leadership

Volunteers were selected to deliver a "pitch" about their institution, department, or group. The large group provided feedback in the context of what was learned so far in the workshop.

SESSION 8: Science in the Public Eye

Facilitator: K.R. Miller, Brown University, Providence, Rhode Island

SESSION 9: Concluding Group Discussion

What did we learn? What did we not learn that we would have liked to learn?

SCOR Retreat

March 15–17

FUNDED BY **The Leukemia & Lymphoma Society**

ARRANGED BY **S. Lowe,** Cold Spring Harbor Laboratory, New York

SCOR (Specialized Center of Research) is a program of the Leukemia & Lymphoma Society. The cornerstone of the SCOR program is its collaborative structure: Every recipient works with a cross-disciplinary team of leading researchers from their own and other universities and medical institutions. In this case, researchers from Cold Spring Harbor Laboratory collaborate with researchers at the Universities of Minnesota, California (San Francisco), and Chicago. This meeting provided an opportunity for those involved to present their data and to interact with one another in person.

OPENING SESSION

J. Zuber, Cold Spring Harbor Laboratory: Leukemia maintenance genes.

SESSION 1: Genetics and Biology of 5q/7q Deletions

M. Le Beau, University of Chicago, Illinois: Commonly deleted intervals in human leukemias with del 5q/7q.
R. Bergerson, University of Chicago, Illinois: Characterization of candidate haploinsufficient genes on chromosome 5.
A. Stoddart, University of Chicago, Illinois: Characterization of the genetic pathways leading to t-MN with a del 5q.
J. Wong, University of California, San Francisco: Modeling 7q deletions in the mouse.
S. Lowe, Cold Spring Harbor Laboratory: Is MLL3 a 7q tumor suppressor?

SESSION 2: Signaling in AML

H. Liu, University of Chicago Hospital, Illinois: Targeting PI3K/mTOR and MEK pathways in AML.
E. Diaz-Flores, University of California, San Francisco: Biochemical profiling of primary leukemias using phosphoflow cytometry.
Z. Zhao, Cold Spring Harbor Laboratory: Ras feedback signaling in leukemogenesis and therapy response.

SESSION 3: Therapy in AML

S. Rathe, University of Minnesota, Minneapolis: This, that, and the other thing.
E. Dolan, University of Chicago, Illinois: Pharmacogenetics of Ara-C.

SESSION 4: Lymphoid Malignancies

M. Dail, University of California, San Francisco: Response and resistance to PI3K inhibition in T-lineage leukemia.
C. Scuoppo, Cold Spring Harbor Laboratory: New tumor suppressor networks in lymphoma.
C. Miething, Cold Spring Harbor Laboratory: Biology of PTEN in lymphoma.

The Third NIMH-Sponsored Brain Camp

March 18–21

FUNDED BY **National Institute of Mental Health**

ARRANGED BY **M. Akil,** National Institute of Mental Health, Bethesda, Maryland
T. Insel, National Institute of Mental Health, Bethesda, Maryland

M. Akil

For the third year, the Banbury Center was very pleased to host the NIMH-sponsored "Brain Camp." The goal of the Brain Camp is to identify areas of neuroscience that are of interest and relevance to psychiatrists and to communicate these to a small group of outstanding psychiatry residents and research fellows. Some of the most distinguished and thoughtful neuroscientists in the country took part in the meeting. The outcome of this meeting will be the start of a neuroscience curriculum that can eventually be shared with psychiatry training programs around the country.

Introduction and Charge: **T. Insel,** National Institute of Mental Health, Bethesda, Maryland

Special Lecture: E. Kandel, Columbia University, New York: Mice, Men, and Mental Illness: Animal Models of Mental Disorders.

SESSION 1: Genetics and Genomics

P. Sklar, Mount Sinai School of Medicine, New York: Toward understanding schizophrenia and bipolar disorder genetics.

J. Gordon, Columbia University, New York: So you've cloned the gene... Now what?

SESSION 2: Developmental Neurobiology

P. Shaw, National Institute of Mental Health, Bethesda, Maryland: In for the long haul: Using longitudinal neuroimaging to understand attention-deficit/hyperactivity disorder.
J. Huang, Cold Spring Harbor Laboratory: Toward a genetic dissection of GABAergic circuits in cerebral cortex: Chandeliers light up the path from genes to cognition.
T. Bale, University of Pennsylvania, Philadelphia: Epigenetics in neurodevelopment: Gene × environment × development × sex.

SESSION 3: Cognitive Neuroscience

C. Carter, University of California, Davis, Sacramento, and A. MacDonald, III, University of Minnesota, Minneapolis: Circuits and symptoms: The cognitive neuroscience of executive control in health and disease.

SESSION 4: Social Neuroscience

K. Pelphrey, Yale University, New Haven, Connecticut: Neural signatures of autism.
T. Insel, National Institute of Mental Health, Bethesda, Maryland: Social neuroscience: A new basic science for psychiatry.

Round Table Discussion with All Speakers: Future Directions in Neuroscience and Psychiatry

SESSION 5: Circuits and Microcircuits

B. Rosen, Massachusetts General Hospital, Charlestown: Multimodal functional neuroimaging.
K. Hong Wang, National Institute of Mental Health, Bethesda, Maryland: Illuminating the functional organization and plasticity of cortical microcircuits.

K. Pelphrey, L. Sjulson

E. Kandel

Curing Melanoma and Other Cancers by Targeted Therapies

March 22–25

FUNDED BY **Hazen Polsky Foundation and the Melanoma Research Alliance**

ARRANGED BY **J. Schlessinger,** Yale University School of Medicine, New Haven, Connecticut
J.D. Watson, Cold Spring Harbor Laboratory, New York

A wealth of genetic and biochemical analyses combined with novel approaches for drug discovery is offering, for the first time, hope for effective new therapies for untreatable cancers such as melanoma. The goal of the meeting was to present new data about targeted therapies that have been recently developed for the treatment of melanoma and other cancers. Genetic and biochemical studies describing novel targets and new approaches for targeted therapies were also described. Finally, molecular mechanisms underlying drug resistance that operate in patients treated with targeted therapies and new approaches for overcoming this problem were reviewed.

S. Topalian, J. Watson

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **J.D. Watson,** Cold Spring Harbor Laboratory, New York

SESSION 1: Genetic and Molecular Basis for Melanoma
Chairperson: M. Herlyn, The Wistar Institute, Philadelphia, Pennsylvania

M. Herlyn, The Wistar Institute, Philadelphia, Pennsylvania: Tumor heterogeneity and the consequences of therapy.

D.E. Fisher, Massachusetts General Hospital, Boston: BRAF-MAPK targets the melanocytic master transcriptional regulator MITF: Targeted therapy and lineage differentiation.

R. Halaban, Yale University School of Medicine, New Haven, Connecticut: Insights from sequencing the melanoma transcriptome and exome.

T. Wiesner, Memorial Sloan-Kettering Cancer Center, New York: Germline mutations in BAP1 predispose to melanocytic tumors.

SESSION 2: Signaling Pathways and Targeted Therapies in Melanoma
Chairperson: J. Schlessinger, Yale University School of Medicine, New Haven, Connecticut

G. Bollag, Plexxikon, Berkeley, California: Discovery of kinase inhibitors for melanoma.
M.J. Weber, University of Virginia, Charlottesville: Compensatory signaling: A mechanism of resistance and a guide to combination therapy.
N. Rosen, Memorial Sloan-Kettering Cancer Center, New York: Resistance to ERK inhibition: Pharmacologic and biologic issues.
R. Marais, Institute of Cancer Research, London, England: Targeting BRAF in melanoma: Synthetic lethality as an approach to treating cells expressing BRAF and other oncogenes.

SESSION 3: Resistance Mechanisms in Melanoma and Other Cancers
Chairperson: B. Stillman, Cold Spring Harbor Laboratory, New York

D.A. Tuveson, CRUK Cambridge Research Institute, Cambridge, United Kingdom: Stromal barriers in pancreatic cancer medicine.
R. Lo, University of California, Los Angeles: Overcoming acquired resistance to BRAF inhibitors.
L.A. Garraway, Dana-Farber Cancer Institute, Boston, Massachusetts: Systematic characterization of resistance to RAF inhibition in melanoma.
P.D. Nisen, GlaxoSmithKline, Collegeville, Pennsylvania: A multifaceted approach to melanoma therapy: BRAF, MEK, MAGE3, and PD-1.

SESSION 4: Epigenetic Pathways in Melanoma and Other Cancers
Chairperson: C.J. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee

E. Bernstein, Mount Sinai School of Medicine, New York: Unraveling the melanoma epigenome.
J.E. Bradner, Dana-Farber Cancer Institute, Boston, Massachusetts: Direct inhibition of epigenetic reader proteins in cancer therapy.
C. Vakoc, Cold Spring Harbor Laboratory: RNAi screening to identify epigenetic vulnerabilities in acute myeloid leukemia.

SESSION 5: Immunotherapies for Melanoma Treatments
Chairperson: J.P. Allison, Memorial-Sloan Kettering Cancer Center, New York

J.P. Allison, Memorial-Sloan Kettering Cancer Center, New York: Immune checkpoint blockade in melanoma therapy.
S. Topalian, Johns Hopkins University School of Medicine, Baltimore, Maryland: Targeting immunological pathways: B7-H1/PD-1 blockade in cancer.
T.F. Gajewski, University of Chicago, Illinois: Regulation of antitumor immunity at the tumor microenvironment.

SESSION 6: Differentiation, Stem Cells, and EMT
Chairperson: H. Varmus, National Cancer Institute, Bethesda, Maryland

K. Struhl, Harvard Medical School, Boston, Massachusetts: Metformin-based combinatorial therapy: New xenograft data on melanoma cell lines.
R. Nusse, Stanford University Medical Center, California: WNT signaling and stem cell control.
R. Kalluri, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Hypoxia-induced epithelial to mesenchymal transition and metastasis.

SESSION 7: Perspectives on Melanoma, Biomarkers, and Targeted Therapies
Chairperson: D.M. Epstein, Astellas–OSI Oncology, Inc., Farmingdale, New York

I. Mellman, Genentech, S. San Francisco, California: The practical empiricist's guide to developing targeted cancer therapies.
D.M. Epstein, Astellas–OSI Oncology, Inc., Farmingdale, New York: Dual IGF-1R blockade in cancer therapy.
J.E. Darnell, The Rockefeller University, New York: STAT 3: Why we must learn to inhibit it and new ideas that might work.

SESSION 8: Animal Models and Melanoma: What Can We Learn?
Chairperson: D.A. Tuveson, CRUK Cambridge Research Institute, Cambridge, United Kingdom

M. McMahon, University of California, San Francisco: Modeling the effects of pathway-targeted therapeutics in genetically engineered mouse models of cancer.

M. Bosenberg, Yale University School of Medicine, New Haven, Connecticut: Optimizing mouse models of melanoma for preclinical testing.

L. Dow, Cold Spring Harbor Laboratory: Using inducible RNAi in vivo to investigate the role of Wnt signaling in melanoma progression.

SESSION 9: Fighting Mesenchymal Cancers
Chairperson: J. Schlessinger, Yale University School of Medicine, New Haven, Connecticut

G.F. Vande Woude, Van Andel Institute, Grand Rapids, Michigan: Targeting c-MET in cancer.

G. Demetri, Dana-Farber Cancer Institute, Boston, Massachusetts: Parsing pathogenetic pathways to accelerate drug development: Universal lessons from GIST and other sarcomas.

R. Sordella, Cold Spring Harbor Laboratory: Intrinsic and extrinsic regulation of metastatic spread of NSCLC.

Closing Remarks and Discussion: J. Schlessinger, Yale University School of Medicine, New Haven, Connecticut

L. Garraway, R. Nusse, H. Varmus

J. Schlessinger

Neuronal Response Variability and Cortical Computation

April 3–6

FUNDED BY **The Swartz Foundation** and **the Gatsby Charitable Foundation**

ARRANGED BY **L. Abbott,** Columbia University, New York
J. Reynolds, Salk Institute for Biological Studies, La Jolla, California

It is becoming increasingly clear that neuronal response variability, in particular low-frequency response correlations, have a profound impact on how populations of neurons encode information and provide an important window into neural circuit function. The goal of the meeting was to bring together experimentalists and theorists seeking to understand neuronal response variability and its implications for cortical computation and to provide a more unified way to think about variability and correlations. Participants examined such questions as Is variability "noise" or is it a signature of important computations that we have yet to understand? How do neural circuits distinguish intrinsic variability in the neural signal from the stimulus-induced variability? How can we best exploit our ability to measure variability and correlations to maximize what we learn about neural circuits?

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **Why Are We Here? L. Abbott,** Columbia University, New York

SESSION 1: Correlations in Data and Models
Chairperson: L. Abbott, Columbia University, New York

A. Kohn, Albert Einstein College of Medicine, Bronx, New York: Signal propagation between V1 and V2.
M. Smith, University of Pittsburgh, Pennsylvania: Structure of neuronal correlation: Depth, distance, and dynamics.
A. Tolias, Baylor College of Medicine, Houston, Texas: Structure of network activity in the neocortex.
A. Renart, Champalimaud Centre for the Unknown, Lisbon, Portugal: Temporal correlations in recurrent neural networks with balanced excitation and inhibition.
J. Curtis, Salk Institute for Biological Studies, La Jolla, California: Changes in neuronal gain modulate neuronal synchronization and correlation.
M. Cohen, Harvard Medical School, Boston, Massachusetts: Using the variability of neuronal populations to compare spatial and feature attention.

SESSION 2: Effects of Stimuli and Attention on Variability and Correlations
Chairperson: H. Sompolinsky, the Hebrew University, Jerusalem, Israel

M. Churchland, Stanford University, California: Stimulus onset quenches neural variability: A widespread cortical phenomenon.
K. Rajan, Princeton University, New Jersey: Stimulus-dependent suppression of chaos in recurrent neural networks.
E. Anderson, Salk Institute for Biological Studies, La Jolla, California: Burstiness and attentional modulation in V4.
T. Pasternak, University of Rochester, New York: Trial–trial variability of cortical neurons reveals the nature of their engagement in a visual discrimination task.
K. Shenoy, Stanford University, California: Toward a single-trial understanding of motor preparation and variability.

SESSION 3: Decisions and Choice Probability
Chairperson: T. Pasternak, University of Rochester, New York

J. de la Rocha, IDIBAPS, Barcelona, Spain: A model for choice probability: Disambiguating whether response variability biases the decision or vice versa.
A. Fontanini, SUNY Stony Brook, New York: Effects of anticipatory cues on gustatory processing in actively sensing rats.
X.-J Wang, Yale University School of Medicine, New Haven, Connecticut: A reservoir of time constants for memory traces in cortical neurons.
M. Shadlen, University of Washington, Howard Hughes Medical Institute, Seattle: Variance as a signature of neural computations during decision making.
D. Ringach, University of California, Los Angeles: Coding by population variance.

SESSION 4: Variability and Correlations in Coding and Circuits
Chairperson: X.-J. Wang, Yale University School of Medicine, New Haven, Connecticut

B. Averbeck, National Institutes of Health, Bethesda, Maryland: Noise correlations and information encoding and decoding.
K. Padmanabhan, Carnegie Mellon University, Pittsburgh, Pennsylvania: Intrinsic biophysical diversity neuronal firing while increasing information content.
M. Corbetta, Washington University School of Medicine, St. Louis, Missouri: Bold signal noise and behavior.
N. Brunel, Université Paris Descartes, France: Response of networks of excitatory and inhibitory neurons to time-dependent inputs.
H. Sompolinsky, the Hebrew University, Jerusalem, Israel: Sensory processing in random cortical networks.

SESSION 5: Variability and Correlation in Visual Processing
Chairperson: J. Reynolds, Salk Institute for Biological Studies, La Jolla, California

Y. Fregnac, CNRS UNIC, Gif sur Yvette, France: Contextual dependency of signal reliability and noise in V1 during sensory processing/adaption of the simple or complex receptive fields to visual statistics.
L. Osborne, University of Chicago, Illinois: Variability in smooth pursuit eye movements and its origin in the brain: Information coding of visual motion in cortical area MT in single units and populations.
V. Dragoi, University of Texas, Houston Medical Center: Correlated variability in laminar cortical circuits.
W. Geisler, University of Texas, Austin: Optimal receptive fields for natural tasks: Efficiency, redundancy, and neural noise.

Closing Remarks and Discussion: J. Reynolds, Salk Institute for Biological Studies, La Jolla, California

V. Dragoi

Lyme Disease in the Proteomics–Genomics Era

April 10–13

FUNDED BY **Time for Lyme, Inc.**

ARRANGED BY **B. Budowle,** University of North Texas Health Science, Fort Worth
S. Schutzer, UMDNJ–New Jersey Medical School, Newark

We are now in the proteogenomic era, characterized by a revolution in high-throughput technologies, most notably in DNA sequencing and protein identification. These new technologies not only do more faster, they enable us to think about doing things differently, to exploit their power to devise new strategies. The goals of this meeting were to explore how new technologies might be used in conjunction with existing techniques, to develop new diagnostic strategies, to forecast promising future tests, and to identify the problems that need to be overcome including supporting biorepositories. Participants included not only those with experience in lyme disease but also a number of individuals whose expertise in proteomics, genomics, and other areas can help move the field forward.

S. Schutzer, A. Hassett

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York

What Do We Hope to Accomplish Here? **S. Schutzer,** UMDNJ–New Jersey Medical School, Newark

SESSION 1: Overview Talks
Chairperson: S. Schutzer, UMDNJ–New Jersey Medical School, Newark

A.C. Steere, Massachusetts General Hospital, Boston: Evolution of lyme disease in the United States covering the clinical spectrum of lyme.

M.E. Schriefer, Centers for Disease Control and Prevention, Ft. Collins, Colorado: Current state of lyme disease laboratory tests and immediate future hopes.

B. Budowle, University of North Texas Health Science, Fort Worth: Things to keep in mind throughout the conference: What if we did have a promising technique, can it be made into a good test and what would be the elements of a good and bad test?

SESSION 2: Animal Models
Chairperson: S. Schutzer, UMDNJ–New Jersey Medical School, Newark

S.W. Barthold, University of California, Davis: Role of animal models in the future.

SESSION 3: Genomic Strategies for Detection and Diagnosis of the Microbe
Chairperson: B. Budowle, University of North Texas Health Science, Fort Worth

D. Ecker, Ibis Biosciences, Inc., Carlsbad, California: Rapid detection of the microbe when culturing is challenging.
P. Chain, Los Alamos National Laboratory, New Mexico: What can we deliver on a large scale?
S. Casjens, University of Utah School of Medicine, Salt Lake City: What has it delivered for lyme disease; is it a game changer?
M. Eshoo, Ibis Biosciences, Inc., Carlsbad, California: Technological methods: Whole-genome amplification in host and vector background. Microbes are unknown.

SESSION 4: Genomics and Proteomics
Chairperson: A.C. Steere, Massachusetts General Hospital, Boston

R.W. McCombie, Cold Spring Harbor Laboratory: What are genomics and sequencing able to deliver in general?
S. Salzberg, University of Maryland, College Park: Bioinformatics of complex genetic data.
T.E. Angel, Pacific Northwest National Laboratory, Richland, Washington: What are integrated strategies to be incorporated into mass spectrometry and protein fractionation? How to maximize the strengths and overcome the challenges.
General Discussion: S. Schutzer, UMDNJ–New Jersey Medical School, Newark, and **T.E. Angel,** Pacific Northwest National Laboratory, Richland, Washington
B. Luft, SUNY Stony Brook, New York: Protein arrays I.
P. Felgner, University of California, Irvine: Protein arrays II.
M. Eshoo, Ibis Biosciences, Inc., Carlsbad, California: Can we identify multiple species and genotypes in ticks and humans?

SESSION 5: Issues for Assay Use and Interpretation on Broad Scale
Chairperson: B. Budowle, University of North Texas Health Science, Fort Worth

M. Lewinski, University of California, Los Angeles: Use in a large clinical laboratory, gold standards, technological and regulatory hurdles.
J. Aucott, Johns Hopkins at Greenspring Station, Lutherville, Maryland: Role of the immune system as a barometer.

SESSION 6: Strategies for Analysis of Genetic Data and Specimen Banks
Chairperson: S. Schutzer, UMDNJ–New Jersey Medical School, Newark

S. Schutzer, UMDNJ–New Jersey Medical School, Newark: Can compartmental genomics and proteomics be used to diagnose a disease and distinguish it from others?
R. Chakraborty, University of North Texas Health Science, Fort Worth: Real-life examples: Getting the right specimens for repository and errors if you fail to do so.
J.S. Fowler, Brookhaven National Laboratory, Upton, New York: PT scan and other methodologies to detect neurological disease.

SESSION 7: Tracking Infection in Animals and Man
Chairperson: S. Schutzer, UMDNJ–New Jersey Medical School, Newark

M. Rusckowski, University of Massachusetts Medical School, Worcester: Can we track the infection as it moves throughout the body?
P. Fox, Animal Medical Center, New York: The dog as a sentinel.
S. Hojvat, Center for Devices and Radiological Health, Rockville, Maryland: Path to FDA clearance.

SESSION 8: Biorepositories and How to Maintain Them and Ensure Their Integrity
Chairperson: A. Hassett, University of Michigan Health System, Ann Arbor

S. Miller, National Museum of Natural History, Washington, D.C.: Past repositories at Smithsonian and future ones for biodefense. Distinctions and defining the purpose.
B. Budowle, University of North Texas Health Science, Fort Worth: Experience with human genetic and microbial repositories.
Closing Remarks and Discussion: S. Schutzer, UMDNJ–New Jersey Medical School, Newark, and **B. Budowle,** University of North Texas Health Science, Fort Worth

Communicating Science

April 29–May 5

FUNDED BY **Boehringer Ingelheim Fonds Foundation for Basic Research in Medicine**

ARRANGED BY **A. Hoffmann,** Boehringer Ingelheim Fonds, Heidesheim, Germany
C. Walther, Boehringer Ingelheim Fonds, Heidesheim, Germany

The Boehringer Ingelheim Fonds has an international program of support for Ph.D. fellowships. It first brought its fellows to the Banbury Center for their annual North American retreat in 2005. It has been a great pleasure to have them return and their 2011 stay at Banbury was the fourth occasion they have been here. At Banbury, they receive intensive instruction in matters such as giving presentations and writing papers, topics usually learned by default (and often poorly) during graduate research.

Opening Remarks: **C. Walther,** Boehringer Ingelheim Fonds, Heidesheim, Germany

SPEAKERS
Chairperson: R. Berwick, Massachusetts Institute of Technology, Cambridge

W. Wells, Global Alliance for TB Drug Development, New York: Writing techniques and how to structure papers. First writing assignment.
B. Tansey, Vanderbilt University Medical Center, Nashville, Tennessee: Presentation of graphic information and how to prepare and deliver a scientific talk.
J. Hudspeth, The Rockefeller University, New York: What makes success in science?

SPECIAL LECTURES

M. Huse, Memorial Sloan-Kettering Cancer Research Institute, New York: Using photoactivation to study cytoskeletal dynamics in lymphocyte activation and inhibition.
S. Webb, National Association of Science Writers, New York: Science in the media.
K. Ris-Vicari, Katie Ris-Vicari Graphic Design, New York, and M. Hansen, *Nature* Publishing Group, New York: How to design figures.
C. Walther, Boehringer Ingelheim Fonds, Heidesheim, Germany: All about BIF.

Antibiotic Resistance: Past, Present, Future

May 15–18

FUNDED BY **Oliver Grace Fund**

ARRANGED BY **J. Davies,** University of British Columbia, Vancouver, Canada
S. Levy, Tufts University School of Medicine, Boston, Massachusetts
J.H. Miller, University of California, Los Angeles
J.D. Watson, Cold Spring Harbor Laboratory, New York

S. Levy, J. Miller, J. Davis

Antibiotics (together with vaccines) are biomedical research's greatest contribution to human health. But the introduction of antibiotics in the early 1940s was accompanied by the development of antibiotic resistance. Initially, resistance occurred by mutation (usually during the course of therapy), but in the 1950s, transmissible resistance was identified. In more recent times, this threat has been magnified because of the emergence of multidrug-resistant clinical strains coupled with the paucity of efforts to find and develop new antibiotics. There is even talk of a return to the pre-antibiotic era. The pharmaceutical industry has tried to keep up with bacterial evolution, but in vain. The need for novel antibiotics and methods of suppressing resistance has never been greater, and this Banbury conference addressed these needs. Participants examined the history of the emergence of antibiotic resistance and the strategies that have been pursued to combat it. This historical background provided a context for discussion of current practical approaches to restoring effective antimicrobial therapy and paths that may prove to be promising in the future.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **J.H. Miller,** University of California, Los Angeles

SESSION 1: Defining the Parameters
Chairperson: J. Davies, University of British Columbia, Vancouver, Canada

J. Davies, University of British Columbia, Vancouver, Canada: A brief history of coevolution of antibiotics and their resistance.
J.H. Miller, University of California, Los Angeles: Antibiotic sensitivities as codrug targets; some consequences of low amounts of antibiotics.
B. Kreiswirth, UMDNJ, Newark, New Jersey: The challenges of XDR-TB.
W. Witte, Robert Koch Institute, Wernigerode, Germany: Spread of livestock-associated MRSA and the risk posed to humans.
G.A. Jacoby, Lahey Clinic, Burlington, Massachusetts: Plasmid-mediated quinolone resistance.

SESSION 2: Ecological Considerations
Chairperson: G. Wright, McMaster University, Hamilton, Canada

G. Wright, McMaster University, Hamilton, Canada: Origins and ecology of resistance: Inhibition of resistance mechanisms.
G. Dantas, Washington University in St. Louis School of Medicine, St. Louis, Missouri: Antibiotic resistome of the commensal microbiota.
R. Kishony, Harvard Medical School, Boston, Massachusetts: The ecology of antibiotic resistance.
A. Tomasz, The Rockefeller University, New York: From resistance gene to the resistant phenotype.
P. Courvalin, Institut Pasteur, Paris, France: Successful resistance determinants are selectively neutral.
C. Thomas, University of Birmingham, United Kingdom: Role of the second eukaryotic-like, isoleucyl tRNA synthetases in a variety of bacteria as a source of resistance to mupirocin and related antibiotics.

SESSION 3: Decline and Fall of β-Lactams
Chairperson: K. Bush, Indiana University, Bloomington

K. Bush, Indiana University, Bloomington: β-Lactamase evolution: Current issues of carbapenemases.
S. Mobashery, University of Notre Dame, Indiana: Molecular mechanism of resistance to β-lactam antibiotics in methicillin-resistance in *Staphylococcus aureus*.
P. Tulkens, Université Catholique de Louvain, Bruxelles, Belgium: Efflux transporters: Impact of patient treatment and role in decreased susceptibility of sparsely used or non-used antibiotics.
O. Lomovskaya, Mpex Pharmaceuticals, San Diego, California: Use of efflux inhibitors as a strategy to overcome and reduce resistance.
H. Zgurskaya, University of Oklahoma, Norman: The assembly and mechanism of multidrug efflux pumps in gram-negative bacteria.

SESSION 4: Biology and Discovery
Chairperson: K. Lewis, Northeastern University, Boston, Massachusetts

K. Lewis, Northeastern University, Boston, Massachusetts: Tolerance, resistance, and opportunities for antibiotic discovery.
B. Eisenstein, Cubist Pharmaceuticals, Lexington, Massachusetts: The antibiotic pipeline: Where it is, what needs fixing.
L. Piddock, University of Birmingham, United Kingdom: Barriers to overcome to get from drug discovery to new antibiotics in patients.
T.R. Walsh, Cardiff University, United Kingdom: The similarities between global antibiotic resistance and global warming: When two worlds collide.
M. Mulvey, National Microbiology Laboratory, Manitoba, Canada: Antimicrobial resistance in Canada.
S. Projan, Medimmune, Gaithersburg, Maryland: Immunoprophylaxis for the prevention of bacterial infections, better than a cure.

SESSION 5: What Is Next?
Chairperson: S. Levy, Tufts University, Boston, Massachusetts

P. Huovinen, University of Turku, Finland: Targeted use of antibiotics: Rapid diagnostics and human microbiota.
S. Levy, Tufts University, Boston, Massachusetts: Circumventing antibiotic resistance: Prevention, not treatment.
E. Kutter, Evergreen State College, Olympia, Washington: Bacteriophages as natural, self-replicating, and self-limiting antimicrobials.
S. Lerner, Wayne State University, Detroit, Michigan: Educating the community about the problem of antibiotic resistance.

NSF Workshop: The Future of Plant Genome Sequencing and Analysis

May 18–20

FUNDED BY **National Science Foundation**

ARRANGED BY **W.R. McCombie,** Cold Spring Harbor Laboratory, New York
M.C. Schatz, Cold Spring Harbor Laboratory, New York
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

In just the past few years, sequencing instrument capabilities have increased more than 1000-fold and are likely to continue to increase about fivefold each year for the next several years. However, analysis methods have not improved nearly as much during the same time period and a variety of technical limitations of these new instruments make it even more difficult to carry out whole-genome sequencing of novel genomes (de novo sequencing). The goals of this meeting were to assess the current state of de novo sequencing, predict what can be expected to develop in the near future, and determine how these exciting technologies could be used to carry out de novo sequencing of entire complex plant genomes.

W.R. McCombie

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York

Challenges and Opportunities in Plant Genomics: Goals of the Meeting: W.R. McCombie, Cold Spring Harbor Laboratory, New York

SESSION 1: Defining the Challenges
Chairperson: W.R. McCombie, Cold Spring Harbor Laboratory, New York

K. Devos, University of Georgia, Athens: The challenges of complex genomes.
D. Neale, University of California, Davis: The nature of the size and complexity of the conifer genome.
R. Ming, University of Illinois, Urbana: Assembly of papaya sex chromosomes.
J. Mudge, National Center for Genome Resources, Santa Fe, New Mexico: Integration of alternative data types.
D. Main, Washington State University, Pullman: Plant community databases: The stewards of knowledge.
J. Romero-Severson, University of Notre Dame, Indiana: Nonmodel, highly heterozygous, outcrossing disomic polyploids with large genomes.

General Discussion and Listing of Key Points

SESSION 2: Technologies
Chairperson: T. Michael, Monsanto Company, Chesterfield, Missouri

D. Schwartz, University of Wisconsin, Madison: Optical mapping and nanocoding systems for genome assembly and analysis.
H. Cao, BioNanomatrix, Inc., Philadelphia, Pennsylvania: High-throughput single-molecule-level imaging of the linear genome for assembly with nanochannel rays.
S. Turner, Pacific Biosciences, Inc., Menlo Park, California: Application of SMRT sequencing to assembly problems.

Discussion and Listing of Key Points

SESSION 3: Assembly
Chairperson: M.C. Schatz, Cold Spring Harbor Laboratory, New York

T. Michael, Monsanto Company, Chesterfield, Missouri: Building tools, pipelines, and processes to utilize long, single-molecule PacBio reads to assemble plant genomes.
A. Zimin, University of Maryland, College Park, Maryland: Efficient assembly of large genomes from short reads.
M. Schatz, Cold Spring Harbor Laboratory: Computational challenges of plant genome assembly.
I. Birol, British Columbia Cancer Research Centre, Vancouver, Canada: Haploid assembly of diploid genomes.
M. Caccamo, Genome Analysis Centre, Norwich, United Kingdom: Assembly of large genomes with cortex.
S. DesChamps, DuPont Experimental Station, Wilmington, Delaware: Local and global de novo assemblies in complex crop genomes.
S. Gnerre, Broad Institute, Cambridge, Massachusetts: Assembling large and small genomes with ALLPATHS-LG.
P. Kersey, European Bioinformatics Institute, Cambridge, United Kingdom: Squeezing through the bottlenecks: Strategies for assembling large genomes.
K. Mockaitis, Indiana University, Bloomington: Using transcriptome data to facilitate genome assembly.
T. Tatusova, National Center for Biotechnology, Bethesda, Maryland: NCBI efforts to support assembly submissions.

General Discussion and Listing of Key Points

Summary Discussion and Future Developments


K. Mockaitis


D. Schwartz

Future of Biomarker Discovery and Biobanks in Cancer Diagnosis, Prognosis, and Therapy

June 23–24

FUNDED BY **SWOG**

ARRANGED BY **L. Baker,** University of Michigan, Ann Arbor
S. Lowe, Cold Spring Harbor Laboratory, New York

The availability of suitable biomarkers to identify appropriate patient populations and tissue-specific drug activation indices is crucial for maximizing the efficacy of many existing and developing therapies and for trial design for drug development. In turn, the successful identification and validation of new biomarkers requires the availability of biospecimens for clinical research. Patient specimens obtained from clinical trials sponsored by NCI-designated cooperative groups such as SWOG provide an incredibly rich source of tissue for the advancement of cancer research, biomarker discovery, and patient care. This meeting examined the optimization of biospecimen repositories as well as the cutting-edge approaches and technologies that may be brought to bear on biomarker discovery.

R. Sordella, S. Powers, S. Lowe

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York

Meeting Expectations and Goals: S. Lowe, Cold Spring Harbor Laboratory, New York
Cooperative Overview and Challenges: L. Baker, University of Michigan, Ann Arbor

SESSION 1: Improving Therapy through Analysis of Clinical Specimens
Chairpersons: H. Varmus, National Cancer Institute, Bethesda, Maryland, and **R. Fisher**, University of Rochester, New York

A. Types of Scientific Questions that Can Be Addressed with Biobank Tissues

T. Tlsty, University of California, San Francisco: Stratifying DCIS biopsies for risk of future tumor formation.
R. Levine, Memorial Sloan-Kettering Cancer Center, New York: Use of clinical trial leukemia samples to gain novel insights into AML pathogenesis.
P. Kantoff, Dana-Farber Cancer Institute, Boston, Massachusetts: Genetic variants in antioxidant pathways and risk of prostate cancer.
R. Sordella, Cold Spring Harbor Laboratory: Intrinsic and extrinsic mechanisms of erlotinib resistance.

B. On the Horizon: New Technologies and Approaches

J. Hicks, Cold Spring Harbor Laboratory: Biomarkers from sequencing, copy number, and single cells.
S. Powers, Cold Spring Harbor Laboratory: Using genomics to develop biomarkers for hepatocellular carcinoma.
A. Van Oudenaarden, Massachusetts Institute of Technology, Cambridge: Single-cell transcript counting in tissue.
L. Wickerham, NSABP Foundation, Pittsburgh, Pennsylvania: Case study.

SESSION 2: How Are We Doing?
Chairperson: T. Tlsty, University of California, San Francisco

A. Lessons from the Cooperatives and Overview of Repositories

S. Hamilton, University of Texas MD Anderson Cancer Center, Houston: The ECOG experience and next-gen clinical trials.
P. Febbo, University of California, San Francisco: Lessons from the cooperative groups and overview of repositories: Cancer and leukemia group B.
W. Franklin, University of Colorado School of Medicine, Aurora: Best practices in cooperative group biobanks: What we have and what we need.

B. Overview of Committees and Selection Process: Who Gets Tissues and How

C. Hoban, SWOG, Ann Arbor, Michigan: Life cycle of biospecimen use in SWOG: Promoting access and overview of distribution process.

C. Ways Cooperative Groups and BioBank Tissue Inform Scientific and Medical Advance

R. Comis, Eastern Cooperative Oncology Group, Philadelphia, Pennsylvania: Role of the cooperative groups in the translational cancer research continuum.
R. Fisher, University of Rochester, New York: LLMPP: A consortium designed to develop a new molecular understanding of lymphoma resulting in targeted therapeutic initiatives.

SESSION 3: Biomarker Discovery Beyond the Cooperative Groups: What Can We Learn?
Chairpersons: P. Febbo, University of California, San Francisco, and **S. Hamilton,** University of Texas MD Anderson Cancer Center, Houston

N. Rosen, Memorial Sloan-Kettering Cancer Center, New York: Dynamic markers of tumor adaptation to therapy.
J. Derry, Sage Bionetworks, Seattle, Washington: The need for sharing data and models.

SESSION 4: Banking Strategies that Facilitate Biomarker Discovery
Chairpersons: N. Rosen, Memorial Sloan-Kettering Cancer Center, New York, and **P. Kantoff,** Dana-Farber Cancer Institute, Boston, Massachusetts

K. Shaw, National Cancer Institute, Bethesda, Maryland: Banking.
W. McCaskill-Stevens, National Cancer Institute, Bethesda, Maryland: Banking of biospecimens from clinical trials: Lessons, challenges, and future opportunities.
N. Ramirez, Nationwide Children's Hospital, Columbus, Ohio: COG and GOG biospecimen resources: More than "tissue bank."
J.-G. Foster, Nationwide Children's Hospital, Columbus, Ohio: Hand in hand: Centralized reference laboratory testing and optimization of cooperative group banking.
S. Paik, NSABP Foundation, Pittsburgh, Pennsylvania: Why great science often fails to become good clinical tests: Development of predictive test for adjuvant Trastuzumab using archived tumor blocks from NSABP trial B-31.

General Discussion: Banking Strategies that Facilitate Biomarker Discovery
Moderator: L. Baker, University of Michigan, Ann Arbor

SESSION 5: Meeting Wrapup: Framing the Future
Chairperson: Scott Lowe, Cold Spring Harbor Laboratory, New York

Discussion

Translation of Cellular and Molecular Mechanisms of Aging to Geriatric Disorders

September 11–13

FUNDED BY **The Therapeutic Strategic Unit of Aging–Sanofi US**

ARRANGED BY **B. Baron,** Sanofi-Aventis, Bridgewater, New Jersey
C. Marta, Sanofi-Aventis, Bridgewater, New Jersey
E. Tamer, Sanofi-Aventis, Bridgewater, New Jersey

The Center and the Laboratory are indebted to members of the CSHL Corporate Sponsor Program, and we are pleased when members of the Program make use of the benefits of the Program. One of these is to hold meetings at Banbury on a topic of their own choosing, and we were happy to welcome members of the Sanofi Therapeutic Strategic Unit of Aging. The meeting brought together key experts with Sanofi scientists to critically evaluate two areas with major implications for aging: (1) the contribution of mitochondrial dysfunction and oxidative stress to aging and its associated disorders and (2) the impact of aging on the immune system and immunosenescence. The focus was on connecting clinical observations to underlying mechanisms, using this knowledge to compare the relevance of potential interventions, and predicting the most appropriate patient populations and outcome parameters.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Keynote Address: **Translating Novel Scientific Findings in Aging into Therapies for Geriatric Practice**
R. Hodes, National Institute on Aging, Bethesda, Maryland

OPENING SESSION: From Biology of Aging to Treatment of Aging Disorders
Chairperson: R. Belder, Sanofi-Aventis, Bridgewater, New Jersey

Introduction of TSU-Aging Strategy and Therapeutic Goals

Opening Remarks: R. Belder, Sanofi-Aventis, Bridgewater, New Jersey

SESSION 1: Mitochondrial Dysfunction, Oxidative Stress, and Translational Interventions
Chairperson: S. Kahn, Gencia Biotechnology, Charlottesville, Virginia

G. Gibson, Weill Medical College, White Plains, New York: The brain's use of glucose and calcium is abnormal in Alzheimer's disease. Why? Does it matter? Can we do anything about it?
R. De Cabo, Biomedical Research Center, NIH, Baltimore, Maryland: Dietary manipulations for healthy aging.
V. Bohr, National Institutes of Health, Baltimore, Maryland: Nuclear and mitochondrial DNA repair defects in aging.
S. Melov, Buck Institute for Research on Aging, Novato, California: A murine mitochondrial model for age-related cardiovascular disease.

Special Lecture
P. Shiels, University of Glasgow, United Kingdom: Cellular and molecular basis of the diseases of aging.

SESSION 1 (*continued*): Mitochondrial Dysfunction, Oxidative Stress, and Translational Interventions
Chairperson: M. Flint Beal, Cornell University, New York

J.M. Cook-Mills, Northwestern University, Chicago, Illinois: Vitamin E isoforms differentially regulate inflammation.
G.S. Shadel, Yale University School of Medicine, New Haven, Connecticut: Regulation of life span by adaptive mitochondrial ROS signaling.
D. Wallace, University of Pennsylvania, Philadelphia: The mitochondrial bioenergetic origin of complex diseases.

SESSION 2: Immunosenescence: Alterations in the Functional and Regenerative Capacity of the Immune System during Immunosenescence. Lessons Learned from Translational Studies
Chairperson: D. Unutmaz, New York University School of Medicine, New York

G. Pawelec, University of Tübingen Clinical School, Germany: Models and mechanisms of human immunosenescence.
B. Grubeck-Loebenstein, Austrian Academy of Sciences, Innsbruck, Austria: Age-related changes in the CD8 T-cell pool and their consequences.

SESSION 2 (*continued*): Immunosenescence: Alterations in the Functional and Regenerative Capacity of the Immune System during Immunosenescence. Lessons Learned from Translational Studies
Chairperson: G. Pawelec, University of Tübingen Clinical School, Germany

D. Unutmaz, New York University, New York: Does chronic inflammation accelerate immunosenescence?
L. Haynes, Trudeau Institute, Saranac Lake, New York: How aging impacts CD4 T cells.
J. Goronzy, Stanford University School of Medicine, California: Receptor threshold calibration in T cells: Implications for T-cell homeostasis and responses with age.
H. Geiger, Cincinnati Children's Hospital Medical Center, Ohio: DC42 activity regulates hematopoietic stem-cell aging and rejuvenation.


D. Wallace


M. Beal

Scientific and Technological Barriers to Global Real-Time Risk Assessment of Vector-Borne Infections

September 18–21

FUNDED BY **Defense Threat Reduction Agency, Department of Defense**

ARRANGED BY **D. Barnard,** Agricultural Research Service, USDA, Gainesville, Florida
R. Breeze, Global Security Directorate, Lawrence Livermore National Laboratory, Livermore, California
D. Fish, Yale University, New Haven, Connecticut
A. Rudolph, Chemical and Biological Defense Directorate, Defense Threat Reduction Agency, Fort Belvoir, Virginia

In 1991, the Banbury Center held its first meeting on lyme disease, then a newly emerging disease. From that first meeting has developed a series of meetings on newly emerging diseases and the threat that they pose to world health. New pathogens emerge regularly in many regions of the world, and they are often zoonotic, making the jump from a wild or domestic animal reservoir or an arthropod vector to humans. This meeting, supported by the U.S. Department of Defense—which has long focused on protecting the health of its forces deployed overseas from numerous infectious diseases not indigenous to the United States—examined what is being done and what could be done to detect such diseases. Questions discussed included What data are available from existing and evolving vector-borne disease surveillance and risk assessment activities and how might these data be enhanced? How might data be generated in low-resource countries, mega cities, and remote regions without long-term and costly investments in human and physical capital? What do we need to know about the ecology of the microbes, vectors, and hosts, including microbial variants that provide early indications of disease activity?

J. Richardson, W. Lipkin, D. Fish

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **A. Rudolph,** Chemical and Biological Defense Directorate, Fort Belvoir, Virginia

SESSION 1: A World of Vector-Borne Threats
Chairperson: R. Breeze, Global Security Directorate, Lawrence Livermore National Laboratory, California

C. Phillips, Texas Tech University, Lubbock: Global disease surveillance, emergent disease preparedness, and national security.
W. Reisen, University of California, Davis: Arbovirus surveillance in California.
D. Fish, Yale University, New Haven, Connecticut: Disease emergence: Lessons from ticks.
G. Glass, Johns Hopkins University, Baltimore, Maryland: Observations on some challenges of finding what you are looking for.
P. Daszak and W. Karesh, EcoHealth Alliance, New York: Predicting the origins and spread of vector-borne diseases.
M. Kilpatrick, University of California, Santa Cruz: A mathematical framework and research plan for predicting which vector-borne pathogens could cause epidemics in North America.
D. Impoinvil, University of Liverpool, United Kingdom: QWeCI and LUCINDA.
D. Knowles, USDA Agricultural Research Service, Pullman, Washington: Determinants contributing to the reemergence of a foreign animal tick-borne infection in the U.S.
T. Myers, Armed Forces Health Sciences Center, Silver Spring, Maryland: The Global Emerging Infections Surveillance and Response System (GEISS).
R. Jarman, Walter Reed Army Institute of Research, Silver Spring, Maryland: Pathogen and disease surveillance: Lessons from dengue cohort studies in northern Thailand.
K. Gage, Centers for Disease Control and Prevention, Fort Collins, Colorado: Selection of sites to evaluate newly developed techniques for assessing global risks of vector-borne infections.

Somalia Scenario and Conference Discussion (Part 1)

SESSION 2: Technologies and Technology Gaps
Chairperson: E. Van Gieson, Chemical and Biological Defense Directorate, Fort Belvoir, Virginia

D. Barnard, USDA Agricultural Research Service, Gainesville, Florida: Detection systems for dipteran vectors.

Discussion led by **W. Reisen,** University of California, Davis
R. Baker, Texas Tech University, Lubbock: The significance of knowing mammalian reservoir species.
T. Briese, Columbia University, New York: Strategies for comprehensive pathogen surveillance and discovery.
I. Lipkin, Columbia University, New York: Microbe hunting.
P. Naraghi-Arani, Lawrence Livermore National Laboratory, California: Novel detection technologies applicable to vector-borne disease surveillance.
M. Eshoo, IBIS Biosciences, Carlsbad, California: The use of PCR/ESI-MS to detect known and novel vector-borne pathogens.

SESSION 3: The Virtual World
Chairperson: B. Knols, Soper Strategies, Amsterdam, the Netherlands

J. Brownstein, Harvard University, Cambridge, Massachusetts: HealthMap: Harvesting informal sources for public health surveillance.
L. Eisen, Colorado State University, Fort Collins: Data management system/decision support system for surveillance and control of vectors and vector-borne diseases.
J. Richardson, Walter Reed Army Institute of Research, Silver Spring, Maryland: Vector map.
B. Knols, Soper Strategies, Amsterdam, the Netherlands: Malariaworld.

Somalia Scenario and Conference Discussion (Part 2)

SESSION 4: Dynamic Spatial Risk Maps
Chairperson: D. Fish, Yale University, New Haven, Connecticut

A. Tatem, University of Florida, Gainesville: Mapping and modeling population and vector-borne infection movements in resource-poor settings.
C. Lord, University of Florida, Gainesville: Scale in models of vector-borne diseases.
B. Blumenthal, Columbia University, New York: Real-time provision of climate analysis for vectoral environment evaluation.
M. Thomson, Columbia University, New York: Climate information for the prediction and prevention of vector-borne diseases.
N. Nurthen, Chemical and Biological Defense Directorate, Fort Belvoir, Virginia: Data sharing/access issues for global real-time risk assessment of vector-borne infections.

Somalia Scenario and Conference Discussion (Part 3)

Strategic Research Initiative for CFS

September 25–27

FUNDED BY **CFIDS Association of America**

ARRANGED BY **K.K. McCleary,** CFIDS Association of America, Charlotte, North Carolina
S. Vernon, CFIDS Association of America, Charlotte, North Carolina

S. Vernon

Banbury Center has held several meetings on chronic fatigue syndrome and related disorders and the Center was pleased to be the site for the inaugural meeting of the Scientific Advisory Board (SAB) of the CFIDS Association of America. During the past few years, CFS research has received high-profile media attention, generating both increased interest and unprecedented opportunities for progress. The Association was looking to its new SAB for guidance on research strategy to encourage innovative research focused on early detection, objective diagnosis, and effective treatment. Banbury takes great pride in the role the Center has had in helping foster research on CFS.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **S. Vernon,** CFIDS Association of America, Charlotte, North Carolina
K.K. McCleary, CFIDS Association of America, Charlotte, North Carolina: 25 Years of Service: Where the CFIDS Association Stands Today

L. Bateman

SESSION 1: Background on the Association and CFS
Chairpersons: R. Silverman, Learner Research Institute, Cleveland, Ohio, and **R. Dodd,** American Red Cross, Holland Laboratory, Rockville, Maryland

A. Lesser, CFIDS Association of America, San Francisco, California: From advocacy to research.
A. Divine, Boulder, Colorado, J. Spotila, King of Prussia, Pennsylvania, and P. Venetucci, Park Ridge, Illinois: Illness narratives.

S. Vernon, CFIDS Association of America, Charlotte, North Carolina: What's in a name?
L. Bateman, The Fatigue Consultation Clinic, Salt Lake City, Utah: A clinical perspective of CFS.
N. Klimas, University of Miami School of Medicine, Florida: Plausible causes/triggers of CFS (as we know it today).
G. Broderick, University of Alberta, Edmonton, Canada: A system biology perspective of CFS.
V. Racaniello, Columbia University Medical Center, New York: Lessons learned from XMRV.

SESSION 2: Transforming CFS Research
Chairpersons: K. Moran, Integrated Strategy, LLC, Greenwood Village, Colorado, and **P. DeStefano,** McDermott Will & Emery, Menlo Park, California

S. Vernon, CFIDS Association of America, Charlotte, North Carolina, and K.K. McCleary, CFIDS Association of America, Charlotte, North Carolina: Strategic and promising areas of research.
E. Aslakson, Centers for Disease Control and Prevention, Atlanta, Georgia: Biomarker hit lists and hypothesis generation.
I. Biaggioni, Vanderbilt University School of Medicine, Nashville, Tennessee: Common platforms for progress.
D. Papanicolaou, Merck Research Laboratories, Kenilworth, New Jersey: Piquing the interest of the pharmaceutical industry.
R. Bromley, Redwood City, California: Not-for-profit research organization successes and failures.
B. Raidt, River Forest, Illinois, and P. Venetucci, CFIDS Association of America, Park Ridge, Illinois: Weaving CFS into the social and medical fabric.

SESSION 3: Transforming Research with the Help of the SAB
Chairperson: K. Frick, CFIDS Association of America, San Francisco, California

R. Bromley, Redwood City, California: Strategic issues: What is next? Strategic governance for research support organizations.

B. Allshouse, L. Bateman

K. Moran

FEBS Journal Editorial Board Meeting

September 30–October 2

FUNDED BY ***FEBS Journal*, John Wiley & Sons, Ltd., New York**

ARRANGED BY **N. Tonks,** Cold Spring Harbor Laboratory, New York
V. Wilkinson, *FEBS Journal*, Cambridge, United Kingdom

The Banbury Center takes a broad perspective on its mission to promote biomedical research. We have, for example, held meetings to discuss scientific fraud, funding for research, and open access journals. This meeting provided an opportunity for the editorial board of the *FEBS Journal* to meet with the journal's staff and discuss the performance and future direction of the journal.

Editorial Office Statistics and Update

V. Johnson and D. Nicholson, Wiley-Blackwell, Oxford, United Kingdom: Wiley-Blackwell presentation and structured brainstorming session.

Editorial Board Meeting

R. Apweiler, European Bioinformatics Institute, Cambridge, United Kingdom
M. Hall, University of Basel, Switzerland
J. Hardy, University College, London, United Kingdom
D. Michele, University of Michigan, Ann Arbor
P. Munoz-Canoves, University Pompeu Fabra, Barcelona, Spain
R. Perham, University of Cambridge, United Kingdom
N. Scrutton, The University of Manchester, United Kingdom
N. Tonks, Cold Spring Harbor Laboratory, New York
A. Wlodawer, National Cancer Institute, Bethesda, Maryland

Dosage, Epigenetics, and the Biology of Hybridization and Hybrids

October 10–13

FUNDED BY **Cold Spring Harbor Laboratory/Pioneer DuPont Joint Collaborative Project**

ARRANGED BY **Z. Lippman,** Cold Spring Harbor Laboratory, New York
R. Martienssen, Cold Spring Harbor Laboratory, New York
R. Williams, DuPont Experimental Station, Wilmington, Delaware

Each year, members of the Cold Spring Harbor–Pioneer DuPont Joint Collaborative Group meet to review the progress of the collaboration. In addition, a small number of researchers not part of the collaboration come to the meeting for discussion of an important topic related to the work of the collaboration. This year, the topic dealt with the molecular biology of hybridization, exploring the roles of gene expression in phenotypic variation—with a particular emphasis on changes not easily explained by simple nucleotide changes (for example, chromosome structure, genome organization, epigenetics, and regulatory RNA). These mechanisms are likely to have effects on plant development, response to stress, heterosis, and evolution.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **Z. Lippman,** Cold Spring Harbor Laboratory, New York

SESSION 1: Genomics and Epigenomics
Chairperson: A. Rafalski, DuPont Experimental Station, Wilmington, Delaware

J.-M. Chia, Cold Spring Harbor Laboratory: HapMap II.
J. Lu and M. Regulski, Cold Spring Harbor Laboratory: Progress on epigenetic variation profiling.
V. Llaca, DuPont Experimental Station, Wilmington, Delaware: The maize methylome pipeline.
M. Dotto, Cold Spring Harbor Laboratory: Small RNAs in lbl1 mutants: Genome-wide search for ta-siRNA loci in maize.
F. Van Ex, Cold Spring Harbor Laboratory: Alternate regulation of RNAi components as an epigenetic basis for apomixis.
A. Eveland, Cold Spring Harbor Laboratory: Systems approaches in maize inflorescence architecture.

SESSION 2: Mechanisms of Reproductive Development
Chairperson: D. Jackson, Cold Spring Harbor Laboratory, New York

C. MacAlister, Cold Spring Harbor Laboratory: Control of meristem formation and homeostasis at the transition to flowering in tomato: Role of terminating flower and fasciated flower.
J. Habben, Pioneer Hi-Bred International, Inc., Johnston, Iowa: Testing of maize transgenics for drought tolerance.
B. Il Je, Cold Spring Harbor Laboratory: New players in maize fasciated pathways and seed row number.
D. Jackson, Cold Spring Harbor Laboratory: A new pathway for meristem maintenance in maize: Uniting fasciated and abphyl phenotypes.
B. Li, DuPont Experimental Station, Wilmington, Delaware: Cloning of maize mutant genes involved in reproductive development.
S. Lawit, Pioneer Hi-Bred International, Johnston, Iowa: Paths toward self-reproducing hybrids.

SESSION 3: Dosage, Development, and Hybridization
Chairperson: Z. Lippman, Cold Spring Harbor Laboratory, New York

Z. Lippman, Cold Spring Harbor Laboratory: Dynamics of meristem maturation and the evolution of inflorescence architecture.
O. Danilevskaya, Pioneer Hi-Bred International, Johnston, Iowa: Functional analysis of the PEBP gene family from maize.
O. Loudet, INRA, Versailles, France: Natural variation for growth and allelic incompatibilities in *Arabidopsis thaliana*.
M. Tanurdzic, Cold Spring Harbor Laboratory: RNAi and inheritance of epigenetic states in plant interspecific hybrids.
L. Comai, University of California, Davis: Dosage-dependent interspecific incompatibility in *Arabidopsis*.
M. Freeling, University of California, Berkeley: Ancient epigenetic origins of genome dominance following paleopolyploidies, and gene regulatory consequences.

SESSION 4: Epigenetics
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory, New York

R. Mosher, University of Arizona, Tucson: Pol IV-dependent siRNAs in *Arabidopsis* seeds.
J. Reinders, DuPont Experimental Station, Wilmington, Delaware: Dynamic retroelement transmission rates during sexual reproduction of *Arabidopsis*.
M. Hudson, University of Illinois, Urbana: The genetics and effects of small RNA expression in *Arabidopsis* and maize hybrids.
R. Martienssen, Cold Spring Harbor Laboratory: Heterochromatin, small RNA, and the epigenetic control of gametogenesis.
M. Timmermans, Cold Spring Harbor Laboratory: Generation of robust development patterns by opposing gradients of mobile small RNAs.
J. Birchler, University of Missouri, Columbia: Studies at the intersection of ploidy and heterosis.

SESSION 5: Tackling Genomics and Phenomics
Chairperson: R. Williams, DuPont Experimental Station, Wilmington, Delaware

M. Schatz, Cold Spring Harbor Laboratory: Challenges and solutions for plant genome assembly.
K. Creasey, Cold Spring Harbor Laboratory: Epigenetically activated small RNA mediates transgenerational phenotypes in *Arabidopsis*.
M. Frank, Pioneer Hi-Bred International, Johnston, Iowa: NUE screens: A chimeric approach.
C. Lu, DuPont Experimental Station, Wilmington, Delaware: Forward genetic screens in drought discovery.
S. Pasternak, J. Stein, and A. Olson, Cold Spring Harbor Laboratory: What up with wheat? Progress on wheat genome sequencing.


D. Ware


M. Freeling


T. Michael

Genotype to Phenotype: Deriving Biological Knowledge from Large Genomic Data Sets

October 16–19

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **T. Michael,** Monsanto Company, Chesterfield, Missouri
P. Schnable, Iowa State University, Ames

In the past few years, sequencing instrument capabilities have increased more than 1000-fold and are likely to continue to increase about fivefold each year for the next several years. However, analysis methods have not improved nearly as much during the same time period, and a variety of technical limitations of these new instruments make it even more difficult to carry out whole-genome sequencing of novel genomes (de novo sequencing). The goals of this meeting were to assess the current state of de novo sequencing, predict what can be expected to develop in the near future, and determine how these exciting technologies could be used to carry out de novo sequencing of entire complex plant genomes.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Goals and Structure of Meeting: **T. Michael,** Monsanto Company, Chesterfield, Missouri, and **P. Schnable,** Iowa State University, Ames

SESSION 1

R. Reiter, Monsanto Company, St. Louis, Missouri: Large breeding data sets.
E. Spalding, University of Wisconsin, Madison: Phenotyping in the laboratory.
T. Harkins, Life Technologies, Carlsbad, California: Future of next-gen sequencing technologies.
L. Comai, University of California, Davis: Sexual incompatability in a genomic context.
R. Flavell, Ceres, Inc., Thousand Oaks, California: Bold ideas for energy crops.
D. Fischoff, Monsanto Company, St. Louis, Missouri: Future opportunities in biotech breeding.

General Discussion and Focus Challenge

SESSION 2

M. Schatz, Cold Spring Harbor Laboratory: Novel genome assembly strategies.
T. Michael, Monsanto Company, Chesterfield, Missouri: Novel genomes.
A. Paterson, University of Georgia, Athens: Comparative genomics.
S. Jackson, University of Georgia, Athens: Economically important crop genomes.
W.R. McCombie, Cold Spring Harbor Laboratory: Strategies for next-gen sequencing.
T. Mockler, Oregon State University, Corvallis: Genome features.

SESSION 3

Moderators: T. Michael, Monsanto Company, Chesterfield, Missouri, and **P. Schnable,** Iowa State University, Ames

General Discussion and Focus Challenge

SESSION 4

Introduction and Day 1 Summary: T. Michael, Monsanto Company, Chesterfield, Missouri

R. Last, Michigan State University, Lansing: Metabolomics.
P. Benfey, Duke Institute for Genome Sciences and Policy, Durham, North Carolina: Transcriptomics.
R. Martienssen, Cold Spring Harbor Laboratory: Epigenomics.
S. Briggs, University of California, San Diego: Proteomics.
I. Baxter, U.S. Department of Agriculture, St. Louis, Missouri: Ionomics.
T. Altmann, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany: Phenomics.

General Discussion and Focus Challenge

SESSION 5

E. Buckler, Cornell University, Ithaca, New York: Genotype to phenotype in cross-pollinated crops.
N. Springer, University of Minnesota, St. Paul: Methylation variation.
P. Schnable, Iowa State University, Ames: Structural variation.
N. Stein, Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany: Genotype to phenotype in self-pollinated crops.
O. Loudet, INRA Versailles, France: Natural variation.
D. Kliebenstein, University of California, Davis: Statistical quantitative genetics.

SESSION 6

Moderators: T. Michael, Monsanto Company, Chesterfield, Missouri, and **P. Schnable,** Iowa State University, Ames

General Discussion and Focus Challenge

SESSION 7

Introduction and Day 2 Summary: P. Schnable, Iowa State University, Ames

V. Walbot, Stanford University, California: Studying development in a genomic context.
S. Goff, University of Arizona, Tucson: Model-based approaches to molecular breeding.
J. Bennetzen, University of Georgia, Athens: Interactions between plant genotypes and soil microflora.
M. Timmermans, Cold Spring Harbor Laboratory: Small RNAs regulating shoot meristem function.

SESSION 8

Moderators: T. Michael, Monsanto Company, Chesterfield, Missouri, and **P. Schnable**, Iowa State University, Ames

General Discussion and Focus Challenge

Metformin and Neoplasia

October 30–November 2

FUNDED BY **Oliver Grace Cancer Fund**

ARRANGED BY **M. Pollak,** McGill University, Montreal, Canada
C. Thompson, Memorial Sloan-Kettering Cancer Center, New York

Metformin is widely prescribed for type II diabetes, and it is known to be a safe and effective agent for this condition. Recent retrospective epidemiologic data suggest that use of metformin or related biguanides is associated with substantially reduced cancer incidence and/or improved cancer outcomes. Furthermore, these compounds have been shown to reduce tumor growth in several in vivo models and to reduce tumor formation in carcinogenesis assays. Participants at this meeting reviewed and critically assessed the most recent results in the field and discussed the many proposed mechanisms of action. The question of potential clinical applications for treatment or prevention was also considered, and gaps in knowledge such as how to optimize pharmacokinetics, identification of predictive biomarkers to select patients who may benefit, and the definition of rational combinations were considered.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **M. Pollak,** McGill University, Montreal, Canada, and
C. Thompson, Memorial Sloan-Kettering Cancer Center, New York

SESSION 1

History

C. Bailey, Aston University, Birmingham, United Kingdom: History of biguanides and development of metformin as an antidiabetic.

Pharmaco-Epidemiology

J. Johnson, University of Alberta, Edmonton, Canada: Epidemiologic evidence for an influence of metformin on neoplasia.

Cancer Models

P. Dennis, National Cancer Institute, Bethesda, Maryland: Metformin as a chemopreventive agent in mouse models of cancer.

M. Martin, Institute of Cancer Research, London, United Kingdom: Investigating the potential for metformin as an antimelanoma agent.

B. Zheng, Columbia University, New York: Cross-talk between LKB1-AMPK and BRAF signaling pathways and its implication for melanoma therapy.

D. Sabatini, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Nutrient sensing by the mTOR pathway.

SESSION 2: Cancer Models

K. Struhl, Harvard Medical School, Boston, Massachusetts: Metformin selectively kills cancer stem cells and acts together with chemotherapy to prolong remission.
F. Bost, INSERM U895, University of Nice Sophia-Antipolis, France: The multiple biological actions of metformin on cancer cells.
M. Schwab, Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, and University Tübingen, Germany: Pharmacokinetics and pharmacogenomics of metformin.

Mechanistic Aspects I: Mitochondria
B. Guigas, Leiden University Medical Center, the Netherlands: Role of mitochondria in the mechanism of action of metformin.
M. Vander Heiden, Dana-Farber Cancer Institute, Boston, Massachusetts: Metabolic pathway regulation in cancer.

SESSION 3: Mechanistic Aspects II: Cellular Physiology

M. Haigis, Harvard Medical School, Boston, Massachusetts: Role of sirtuins in metabolism.
D. Shackelford, University of California, Los Angeles: Phenformin as a cancer therapeutic.
G. Hardie, University of Dundee, United Kingdom: AMPK:A target for metformin that is down-regulated in cancer cells and during viral infection.
G. Rocha, Universidade Estadula de Campinas, Sao Paulo, Brazil: Metformin and chemotherapy.
A. Selvaraj, University of Cincinnati, Ohio: Metformin and TOR pathway.
N. Sonenberg, McGill University, Montreal, Canada: Metformin alters the translatome.
I. Topisirovic, McGill University, Montreal, Canada: Effects of biguanides and mTOR inhibitors on protein synthesis and energy metabolism.

SESSION 4: Mechanistic Aspects II: Cellular Physiology (*continued*)

C.L. Walker, MD Anderson Cancer Center, Smithville, Texas, and J. Pouyssegur, University of Nice, France: Targeting lactic acid export of glycolytic tumors. Best anticancer benefit: Metformin or AMPK block?
F. Wondisford, Johns Hopkins University, Baltimore, Maryland: Molecular mechanism of metformin action.
B. Viollet, Institut Cochin Université Paris Descartes, France: Metformin actions in the liver to inhibit gluconeogenesis: Insights to possible mechanisms relevant to oncology.
M. Driscoll, Rutgers University, Piscataway, New Jersey: Metformin engages pathways that promote healthy aging in *C. elegans*.
M. Pollak, McGill University, Montreal, Canada: Metformin can influence tumor growth as an indirect consequence of actions on the liver.
G. Hardie, University of Dundee, United Kingdom: Relationship between ATM and metformin action.

Early Clues from the Clinic
G. Hardie, University of Dundee, United Kingdom: Results of biomarker trial in breast cancer.

SESSION 5: Early Clues from the Clinic

S. Jiralerspong, Baylor College of Medicine, Houston, Texas: Clinical evidence regarding metformin antineoplastic activity.

Novel Aspects
G. Ferbeyre, University of Montreal, Canada: Metformin reduces DNA damage and mutations due to endogenous reactive oxygen species.
R. Jones, McGill University, Montreal, Canada: Metformin effects on immunity of possible relevance to oncology.

Repurposing Drugs
A. So, Duke University, Durham, North Carolina: Repurposing drugs: Policy challenges and economic prospects.

Concluding Remarks and General Discussion: M. Pollak, McGill University, Canada, and **C. Thompson,** Memorial Sloan-Kettering Cancer Center, New York

Workshop: The Future of the Epigenomics of Plants International Consortium

November 2–4

FUNDED BY **Epigenomics of Plants International Consortium**

ARRANGED BY **R. Martienssen,** Cold Spring Harbor Laboratory, New York
C. Pikaard, Indiana University, Bloomington
D. Wagner, University of Pennsylvania, Philadelphia

Plant research is leading the way in many areas of epigenomics, and the Epigenomics of Plants International Consortium (EPIC) is a National Science Foundation (NSF)-funded research coordination initiative to form an international consortium to decipher the plant epigenome. It has been clear for many years that epigenetic interactions with the environment shape the plant body plan during development and control growth and survival responses of these sessile organisms. As a result, plants have a sophisticated epigenomic "toolkit" that modulates genome accessibility. Deciphering the plant epigenome is a large task and will be most effectively achieved via an internationally coordinated effort. This conference discussed the intellectual questions, transformative methodologies, and infrastructure needs required to achieve this goal, as well as the means to engage funding agencies and the international research community as a whole.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Overview of EPIC: **D. Wagner,** University of Pennsylvania, Philadelphia: Our mandate, relevance to recent U.S. Plant Science Summit and 10-year goals, EPIC's accomplishments to date, challenges ahead.

SESSION 1: Defining the Mission
Concurrent meeting of breakout groups led by C. Pikaard (Group A) and D. Wagner (Group B)

Group A: Potential grand challenges, gaps in our understanding or capabilities, long- and short-term goals.
Group B: Epigenetic modifications to be examined, what "reference epigenomes" are needed, model systems vs. crops, conditions (e.g., developmental, environmental, and genetic) to be analyzed. Report of breakout group A and full group discussion.
Report of breakout group B and full group discussion.

SESSION 2: Obtaining, Storing, and Displaying the Data
Concurrent meeting of breakout groups led by C. Pikaard (Group A) and R. Martienssen (Group B)

Group A: Data sets and tools needed.
Group B: Data collection (coordination, standards, and analysis), data storage, data display, and availability.
Report of breakout group A and full group discussion.
Report of breakout group B and full group discussion.

SESSION 3: Plan of Action
Concurrent meeting of breakout groups led by D. Wagner (Group A) and R. Martienssen (Group B)

Group A: Engaging the community.
Group B: Governance structure for an international EPIC consortium.
Report of breakout group A.
Report of breakout group B.
Full group discussion of the White paper draft: Contents, authors.
Next steps for EPIC, future meetings, and engaging the funding agencies for an international cooperative effort.

L. Dennis

E. Richards, C. Pikaard

V. Chandler

Myc and the Pathway to Cancer

November 6–9

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **C. Dang,** University of Pennsylvania, Philadelphia
R. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, Washington

Myc was discovered 30 years ago and was recognized as being a key player in cancer development. Although a great deal has been learned about Myc interactions and functions, we still lack a detailed understanding of how Myc activity influences normal versus cancer cell behavior. Specific topics covered at this meeting included the regulation of *myc* gene transcription, the role of Myc in normal and tumor stem cells, transcriptional and nontranscriptional activities of Myc, and functions of the extended Myc network (including Mlx and Mondo proteins). Participants included experts on cancer models who provided perspective concerning potential roles of Myc in different tumor systems. The meeting explored ways through which *myc*, as a pivotal oncogene in human cancers and its associated pathways, can provide targets for the development of therapies.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **R. Eisenman,** Fred Hutchinson Cancer Research Center, Seattle, Washington

SESSION 1: Myc Transcriptional Activities
Chairperson: C. Dang, University of Pennsylvania, Philadelphia

R. Young, Whitehead Institute, Boston, Massachusetts: Control of gene expression by c-Myc.

S. Hann, Vanderbilt University, Nashville, Tennessee: ARF inhibition of c-Myc transcriptional domain ubiquitylation controls c-Myc-mediated apoptosis.

M. Cole, Dartmouth University, Lebanon, New Hampshire: A new mechanism for Myc-mediated repression.

SESSION 2: Regulation of Myc Abundance
Chairperson: S. Hann, Vanderbilt University, Nashville, Tennessee

D. Levens, National Cancer Institute, Bethesda, Maryland: How a simplified model of Myc function requires and explains the complexity of the c-Myc promoter.

J. Bradner, Dana-Farber Cancer Institute, Boston, Massachusetts: Chemical inhibition of Myc expression and function.

R. Sears, Oregon Health & Science University, Portland: Phosphorylation and proline isomerization events that regulate c-Myc DNA binding, oncogenetic activity, and protein stability.
W. Tansey, Vanderbilt University, Nashville, Tennessee: Regulation of Myc by the ubiquitin-proteasome system.
J. Freedman, GlaxoSmithKline, Collegeville, Pennsylvania: Regulation of Myc through bromodomain modulation (ready for patient testing).

SESSION 3: Myc Regulation of Cell Physiology
Chairperson: L. Penn, University of Toronto, Ontario, Canada

G. McArthur, Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia: Inhibition of RNA polymerase I as a therapeutic strategy for cancer-specific activation of p53 in Myc-driven malignancy.
L. Johnston, Columbia University, New York: Myc, p53, and metabolism: A *Drosophila* model for premalignancy.
C. Dang, University of Pennsylvania, Philadelphia: Regulation of cancer cell metabolism by Myc and therapeutic targets.
D. Ayer, University of Utah, Salt Lake City: Nutrient sensing by MondoA:M1x complexes.

SESSION 3 (*continued*): Myc Regulation of Cell Physiology
Chairperson: M. Cole, Dartmouth University, Lebanon, New Hampshire

S. McMahon, Thomas Jefferson University, Philadelphia, Pennsylvania: Control of the survival/apoptosis decision by Myc.
B. Amati, Italian Institute of Technology, Milan: Targeting the ATR-Chk1 pathway in Myc-induced lymphoma.
S. Dalton, University of Georgia, Athens: Myc in pluripotency and reprogramming.

SESSION 4: Myc-Regulated Targets and Pathways
Chairperson: C. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee

J. Sedivy, Brown University, Providence, Rhode Island: Role of Myc in aging: The best of two worlds.
S. Lowe, Memorial Sloan-Kettering Cancer Center, New York: Characterizing tumor maintenance genes using mouse models and RNAi.
L. Staudt, National Cancer Institute, Bethesda, Maryland: Pathogenetic pathways and treatment strategies from a Burkitt lymphoma genome project.
M. Henriksson, Karolinska Institutet, Stockholm, Sweden: MYCN and neuroblastoma differentiation.
D. Felsher, Stanford University, California: Multiscale modeling of Myc-associated oncogene addiction.

SESSION 5: Tumorigenesis and Therapeutic Approaches
Chairperson: M. Roussel, St. Jude Children's Research Hospital, Memphis, Tennessee

L. Penn, University of Toronto, Ontario, Canada: Strategies to target Myc as an effective anticancer therapeutic: Understanding posttranslational modifications as a point of Myc regulation.
M. Eilers, University of Würzburg, Germany: Synthetic lethal interactions with deregulated Myc or genome-wide analyses of Myc and miz1-binding sites
M. Ptashne, Memorial Sloan-Kettering Cancer Center, New York: Epigenetic switches are easier to construct in eukaryotes than in prokaryotes.

SESSION 5 (*continued*): Tumorigenesis and Therapeutic Approaches
Chairperson: M. Eilers, University of Würzburg, Germany

T. Look, Dana-Farber Cancer Institute, Massachusetts: Apoptotic response in MycN-overexpressing sympathoadrenal cells is blocked by activated ALK, leading to neuroblastoma.
C. Grandori, Fred Hutchinson Cancer Research Center, Seattle, Washington: Identification of therapeutic targets for Myc-driven cancers by functional genomics.
R. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, Washington: A point mutation in Myc generates a tumor-prone phenotype.
M. Roussel, St. Jude Children's Research Hospital, Memphis, Tennessee: Myc proteins in pediatric medulloblastoma.
C. Vakoc, Cold Spring Harbor Laboratory: RNAi screening to identify epigenetic vulnerabilities in acute myeloid leukemia.

Concluding Remarks and General Discussion: R. Eisenman, Fred Hutchinson Cancer Research Center, Seattle, Washington, and **C. Dang,** University of Pennsylvania, Philadelphia

Transmissible Amyloidoses

November 29–December 2

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J. Collinge,** University College London, United Kingdom
D. Goldgaber, SUNY Stony Brook, New York

After succeeding in demonstrating the transmission of kuru and Creutzfeldt–Jakob disease (CJD) in the late 1960s, Carleton Gajdusek suspected that other late-onset diseases of the brain might also be transmissible. In the 1970s, he inoculated hundreds of animals with brain tissues of patients with Alzheimer's disease, multiple sclerosis, Parkinson's disease, and other diseases and kept them under observation for many years, some of them for decades. Using the same criteria that worked for kuru and CJD, namely, clinical symptoms and gross pathological changes in the brain, he found not a single case of transmission. In the 1990s, however, data began to accumulate that Alzheimer's disease amyloidosis can be transmitted to primates, and transmission of β-amyloid to transgenic mice was demonstrated. The time was clearly right for a critical review of the data on transmission of the amyloidoses, the mechanisms involved, and the implications for human health.

D. Goldgaber, J. Collinge

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **D. Goldgaber,** SUNY Stony Brook, New York

SESSION 1: Mammalian and Yeast Prions and Their Properties
Chairperson: J. Collinge, University College London, United Kingdom

J. Collinge, University College London, United Kingdom: Mammalian prion propagation, strains, and transmission barriers.
B. Caughey, NIAID Rocky Mountain Laboratories, Hamilton, Montana: Structure and detection of prions.
I. Vorberg, Deutsches Zentrum für Neurodegenerative Erkrankugen, Bonn, Germany: Propagation of yeast prions in mam- malian cells.
R. Wickner, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland: Yeast prion disease amyloid structure can explain prion strains.
C. Weissmann, Scripps Research Institute, Jupiter, Florida: Mutation of prions.

J. Buxbaum, C. Weissmann

D. Eisenberg, I. Vorberg

SESSION 2: Mechanisms of Protein Misfolding/Aggregation and Structure of Amyloids
Chairperson: A. Horwich, Yale University School of Medicine, New Haven, Connecticut

R. Tycko, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland: In vitro vs. in vivo β-amyloid fibril structures: Propagation of amyloid structures in vitro.

D. Eisenberg, University of California, Los Angeles: Are structural polymorphisms the basis of prion strains?

R. Morimoto, Northwestern University, Evanston, Illinois: Transmission of protein aggregates in *C. elegans*.

SESSION 3: Alzheimer's Disease and Tauopathies
Chairperson: D. Goldgaber, SUNY Stony Brook, New York

D. Goldgaber, SUNY Stony Brook, New York: Historical perspective and review of archived NIH primate transmission series.

A. Nicoll, UCL Institute of Neurology, London, United Kingdom: Review of archived attempted transmission of Alzheimer pathology to transgenic mice.

L. Walker, Emory University, Atlanta, Georgia: Seeded induction of β-amyloid deposition in transgenic rodents.

M. Jucker, University of Tübingen, Germany: Characterization of β-amyloid-inducing seed.

M. Goedert, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Prion-like properties of assembled tau.

C. Soto, University of Texas Medical School, Houston: Transmission of Alzheimer's disease and type-2 diabetes.

M. Diamond, Washington University, St. Louis, Missouri: Propagation of protein misfolding in neurodegenerative diseases.

General Discussion

SESSION 4: Cellular Spread and Pathogenesis: Prion-Like Mechanisms
Chairperson: J. Buxbaum, Scripps Research Institute, La Jolla, California

A. Bertolotti, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Prion-like propagation of mutant SOD1 misfolding.

S.-J. Lee, Konkuk University, Seoul, Korea: Transmission of synucleinopathies and neuroinflammation via extracellular α-synuclein.

K. Luk, University of Pennsylvania School of Medicine, Philadelphia: α-Synuclein transmission in synucleinopathies.

T. Outeiro, University Medizin Göttingen, Germany: Intra- and extracellular effects of α-synuclein oligomers: Implications for transmission.

General Discussion

SESSION 5: Transmissibility of Other Amyloidoses
Chairperson: D. Eisenberg, University of California, Los Angeles

J. Watts, University of California, San Francisco: Bioluminescence imaging of induced protein deposition in transgenic mice.

J. Buxbaum, Scripps Research Institute, La Jolla, California: Amyloid–amyloid interactions.

K. Higuchi, Shinshu University School of Medicine, Matsumoto, Japan: Transmission of amyloidoses in mouse and cheetah: Implications in human systemic amyloidoses.

P. Westermark, Uppsala University, Sweden: Transmission of systemic amyloidosis/dissemination of deposits in systemic amyloidoses.

General Discussion

L. Walker, M. Jucker

Evolution of Neural Circuits and Behavior

December 7–9

FUNDED BY **Marie Robertson Memorial Fund**

ARRANGED BY **K. Honegger,** Cold Spring Harbor Laboratory, New York
S. Shea, Cold Spring Harbor Laboratory, New York

Nervous systems have evolved under unique sets of selective pressures driving adaptive changes, and thus, their structure and function reflect the organism's environment. The ultimate expression of these forces on the brain is diversity of behavior. Empirical studies of the interaction among environment, neural processing, and behavior have largely consisted of neuroethological, comparative, and molecular genetic approaches. Participants in this meeting discussed how the synthesis of these complementary approaches might enrich our understanding of nervous system function and further aid in the interpretation of experimental findings across animal phyla.


D. Chklovskii

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **K. Honegger,** Cold Spring Harbor Laboratory, New York

SESSION 1: Fundamental Constraints of the Evolution of Neural Circuits
Chairperson: D. Chklovskii, Janelia Farm/HHMI, Ashburn, Virginia

S. Grant, University of Edinburgh, United Kingdom: Synapse evolution.
D. Chklovskii, Janelia Farm/HHMI, Ashburn, Virginia: Do more neurons make you smarter? A sensory processing perspective.
J. Niven, University of Cambridge, United Kingdom: Energetic and biophysical constraints on neural circuits.
P. Katz, Georgia State University, Atlanta: Homology and homoplasy in neural circuits underlying behavior.

SESSION 2: Molecular Genetics and the Evolution of Neural Circuits
Chairperson: J. Dubnau, Cold Spring Harbor Laboratory, New York

D. Stern, Howard Hughes Medical Institute, Ashburn, Virginia: Tools and approaches for studying the evolutionary genetics of behavior in closely related *Drosophila* species.
J. Huang, Cold Spring Harbor Laboratory: Chandelier cell: An entry point to cortical circuit organization, assembly, and evolution.
J. Dubnau, Cold Spring Harbor Laboratory: A microRNA–dopamine receptor genetic module in distinct neural circuits for olfactory arousal and olfactory memory.

SESSION 3: Comparative Neuroanatomy
Chairperson: H. Karten, University of California, San Diego

S. Farris, West Virginia University, Morgantown: Evolution of structural and functional novelty in insect mushroom bodies.
C. Ragsdale, University of Chicago, Illinois: Sensory, motor, and memory circuitries in the octopus CNS.
M. Hale, University of Chicago, Illinois: Biomechanical and neural analysis of the evolution of motor systems.
H. Karten, University of California, San Diego: Cells, circuits, and systems: Natural selection and conservation among amniotes.

SESSION 4: Comparative Physiology
Chairperson: H. Zakon, University of Texas, Austin

E. Fortune, Johns Hopkins University, Baltimore, Maryland: Evolution of neural circuits for cooperative behaviors.
N. Sawtell, Columbia University, New York: Mechanisms for predicting sensory events in cerebellum-like circuits.
H. Zakon, University of Texas, Austin: Electric fish are green: Conserving energy and recycling ion channels.
D. Soares, University of Maryland, College Park: Sensory specialization and adaptation in cavefishes.
D. Kelley, Columbia University, New York: Evolution of songs and their neural circuits in *Xenopus*.
C. Fernando, University of Sussex, Brighton, United Kingdom: Darwinian neurodynamics.

General Discussion and Summary: S. Shea, Cold Spring Harbor Laboratory, New York

M. Hale

H. Zakon

Psychiatric Genomics

December 11–14

FUNDED BY **The Stanley Research Foundation**

ARRANGED BY **W.R. McCombie,** Cold Spring Harbor Laboratory, New York
M. Owen, Cardiff University, United Kingdom

The first Banbury Center meeting on molecular human genetics was held in 1982 at a time when restriction-fragment-length polymorphisms (RFLPs) linked to human genetic diseases were first being sought and a complete human gene had yet to be sequenced. How times have changed! And yet it is still difficult to find the genes underlying psychiatric and other complex disorders. However, new high-throughput DNA sequencing techniques have made, or are about to make, it possible to sequence the whole exomes and genomes of large numbers of individuals. This will provide opportunities to develop new gene-hunting strategies for complex genetic disorders. This meeting brought together experts to critically assess current strategies and to outline how genome-scale sequencing can be used most effectively and efficiently.

Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **W.R. McCombie,** Cold Spring Harbor Laboratory, New York

SESSION 1: Common Variants
Chairperson: D. Goldstein, Duke University, Durham, North Carolina

O. Andreassen, Oslo University Hospital, Norway: LD-based annotation enrichment reveals new schizophrenia genes.

P. Gejman, North Shore University Health System–Research Institute, Evanston, Illinois: Genome-wide gene expression study of a schizophrenia GWAS data set.

S. Leal, Baylor College of Medicine, Houston, Texas: Quantifying missing heritability and estimating genetic effects for complex traits due to rare variants.

M. Noethen, University of Bonn, Germany: NCAN in psychiatric disorders: From genetic association to mouse model.

A. Corvin, St. James Hospital, Dublin, Ireland: Schizophrenia redefined: The promise of genomics.

M. Daly, Massachusetts General Hospital, Boston: Autism sequencing?

SESSION 2: Phenotype
Chairperson: D. Goldstein, Duke University, Durham, North Carolina

K. Kendler, Virginia Commonwealth University, Richmond: Genetics and psychiatric nosology in the genomics era.
D. Blackwood, University of Edinburgh, United Kingdom: Phenotypic spectrum of DISC1 variation.
M. Owen, Cardiff University, United Kingdom: Rethinking psychiatric phenotypes.
K. Mitchell, Trinity College, Dublin, United Kingdom: Rare mutations, oligogenic interactions, and endophenotypes.
P. Thomson, University of Edinburgh Centre for Molecular Medicine, United Kingdom: Next-generation sequencing of the *DISC1* (*disrupted in schizophrenia 1*) locus.

SESSION 3: Rare Variants
Chairperson: W.R. McCombie, Cold Spring Harbor Laboratory, New York

W. Byerley, University of California, San Francisco: Whole-genome sequencing of multiplex bipolar pedigrees.
C.T. Caskey, Baylor College of Medicine, Houston, Texas: Whole-genome sequencing of families with multiple affected individuals with schizophrenia.
E. Eichler, University of Washington, Seattle: New mutations and autism.
D. Goldstein, Duke University, Durham, North Carolina: Finding schizophrenia risk factors in sequence data.
M.-C. King, University of Washington School of Medicine, Seattle: Discovering the (many) genes responsible for schizophrenia: Strategies based on genomic sequencing.
J. Lupski, Baylor College of Medicine, Houston, Texas: Reciprocal CNV, mirror image phenotypes, and neuropsychiatric traits.
S. McCarroll, Harvard Medical School, Boston, Massachusetts: What constitutes sufficient evidence that a gene is actually related to schizophrenia?
F. McMahon, National Institute of Mental Health, Bethesda, Maryland: Sequencing members of virtual pedigrees drawn from inbred founder populations.
M. O'Donovan, Cardiff University School of Medicine, United Kingdom: Synaptic genes and de novo mutations.
A. Need, Duke University, Durham, North Carolina: Next-generation sequencing of 170 schizophrenia patients.
A. Palotie, Wellcome Trust Sanger Institute, Cambridge, United Kingdom: Identification of low-frequency variants in the neurodevelopmental studies of the UK10K project.
J. Potash, University of Iowa, Iowa City: Whole-exome sequencing in bipolar disorder.
S. Purcell, Massachusetts General Hospital, Boston: Exome sequencing in schizophrenia.
G.A. Rouleau, CHU Sainte-Justine, Montreal, Canada: De novo mutations in psychiatric diseases.
M. Wigler, Cold Spring Harbor Laboratory: Role of de novo mutation in autism.

SESSION 4: What's Next?
Chairperson: M. Owen, Cardiff University, United Kingdom

F. Henn, Cold Spring Harbor Laboratory: Problems, circuits, genes, and models: An agnostic's thoughts on psychiatric genetics.
T. Lehner, National Institute of Mental Health, Bethesda, Maryland: The neuropsychiatric disease consortium.
W.R. McCombie, Cold Spring Harbor Laboratory: Case controls or family studies, and where we have to go next to understand rare variants.
D. Weinberger, National Institute of Mental Health, Bethesda, Maryland: From gene to brain circuits and back.


Y. Berstein


M.C. King


C.T. Caskey

DNA LEARNING CENTER

DNA LEARNING CENTER
EXECUTIVE DIRECTOR'S REPORT

Preparing students and families to thrive in the gene age

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On December 23rd, our effort in computer *BioMedia* was recognized with the *Science Magazine* Prize for Online Resources in Education. The prize essay, "Lessons from a Science Education Portal," gave us an opportunity to look back on our success in the world of online publishing. When we launched the DNA Learning Center (DNALC) website (www.dnalc.org) in 1996, we could not foresee that it would become an Internet portal with 21 websites, a *YouTube* channel, and smartphone/tablet apps that reached 7.45 million visitors in 2011. The evolution of our online efforts and the challenges we faced along the way provide four lessons for science organizations that want to build modern learning resources.

Lesson 1: Make Content More Visible to Search Engines. After a decade of continuous growth, visitation to our websites mysteriously declined in 2007. During that period, the number of active websites had increased 10-fold, to more than 100 million. In an exponentially crowded web, Google and other search engines had become the de facto arbiters of web visitation. Search engines create an index of the information at a site to scan for search term matches. A "robot" or "spider" visits a website periodically to rebuild its index. Our downturn came as Google changed its search algorithms and we lost "attention" of its robots.

We therefore embarked on an ambitious program to redesign our websites to increase "visibility" to search engines, a process called search engine optimization (SEO). This included directing robots to rich keywords and detailed descriptions of all content, especially animations and videos that cannot be indexed themselves. We also "freshened" content with blogs and newsfeeds. The SEO makeover of dnalc.org nearly doubled average monthly visits from search engine robots, resulting in a 25% increase in visitation in 2010. Continued attention to SEO is keeping us on an upward trajectory.

Lesson 2: Disaggregate and Flow Content through Multiple Channels. Everyone likes a good story, but students and teachers most often turn to the Internet for answers to specific questions or to il-

lustrate key points. These are much harder to find when deeply embedded within a website. Thus, we disaggregated our websites into searchable content "atoms" that can be accessed individually. Greatly aided by our participation in the National Science Digital Library, we have cataloged more than 5300 animations, videos, photos, and illustrations. Eight of 10 visits to dnalc.org now arrive at a content atom identified by an Internet search, often with links to related DNALC items. Moving content to *YouTube* and smartphone apps generated new audiences for DNALC multimedia, contributing 15% of total Internet visits in 2011. Views of 190 high-quality animations and videos on our *YouTube* channel increased 150% to 693,917. *3D Brain*, an app adapted from the *Genes to Cognition* (*G2C*) *Online* website, was downloaded 440,073 times, and the new *Gene Screen* app was downloaded 4888 times.

Lesson 3: Try to Answer the Difficult Question. Every educator and multimedia developer is faced from time to time with the following difficult question from a board member or funding agency: "This program is very nice, but can you prove it actually helps students to learn?" In recent years, Congress has asked the same question of the educational grant portfolios of the National Science Foundation (NSF) and National Institutes of Health (NIH). This has led to increasing pressure on principal investigators to go beyond anecdotal reports of teacher satisfaction with new resources to sophisticated studies of how those resources impact students in the classroom.

We accepted this charge in 2011, conducting classroom experiments with 662 high school and college students to see if the DNALC's *G2C Online* and *Inside Cancer* websites improve science learning. To control for differences between teachers and students, we used a crossover repeated-measures design in which each student participated as both an experimental and control subject, using a DNA website to learn one topic and using lectures, textbooks, or other websites to learn another topic. Strikingly, scores on quizzes given after each topic were significantly higher when students used *G2C Online* (81% vs. 70%) and *Inside Cancer* (85% vs. 73%). Thus, we now have a practical and supportable answer to that difficult question: An engaging website can potentially increase student learning by about one letter grade!

Lesson 4: Help Distribute Cybertools and Data to a Broader Audience. Science takes place on a continuum between research and education. Traditionally, access to limited data kept most good science far to the research end of the spectrum. Now, the ready availability of nearly unlimited data from high-throughput DNA sequencers—plus powerful bioinformatics analyses from shared servers—promises to unify the research-education continuum into a single endeavor. For the first time in the history of science, students and teachers can work with the same data, at the same time, and with the same tools as elite-level researchers. We have devoted considerable effort to developing educational resources to help students generate, share, and analyze the same genome data as scientists.

In 1998, we developed the first cyberexperiment to allow students to analyze a short DNA sequence from their own genome, along with the first DNA database and bioinformatics interface for education (*BioServers*). Our interest in community workspaces and bioinformatics culminated in our involvement in the *iPlant Collaborative*, a project to develop a cyberinfrastructure for plant science research. As educational outreach for this project, we are developing a parallel bioinformatics workflow, *DNA Subway* (www.dnasubway.org), for genome analysis and comparison. These projects begin to push bioinformatics mid spectrum, where the battle for advanced cyberliteracy must take place. Here, we all must work hard to make bioinformatics workflows accessible to the legions of bright biologists who are not computer scientists and who do not know one down the hall.

A Trio of New *BioMedia* Projects

We partnered with the Victor Center for the Prevention of Jewish Genetic Diseases, at Einstein Healthcare Network, to produce a new iPhone and iPad app, *Gene Screen*. The animated app aims

to help people understand that (1) the prevalence of recessive genetic diseases varies among different population groups and (2) healthy members of at-risk groups should consider being screened to see if they are carriers of a disease gene. Interactive Punnett squares update this classic way to calculate how traits are passed from parents to children, and a "slot machine" spins to show carrier frequencies of 19 genetic diseases in Jewish communities versus the general population. An interactive ancestry map highlights recessive genetic diseases that are more common in certain regions of the world. Although the app was specifically designed to alert people to genetic screening, precollege science teachers and students will appreciate this fun way to learn basic genetics.

The Spinal Muscular Atrophy (SMA) Foundation has long supported CSHL researcher Adrian Krainer's work on RNA splicing and molecular approaches to SMA therapies. To coincide with the first clinical trials of a drug based on Dr. Krainer's methods, we developed the Internet microsite *Learn About SMA* as a resource for patients, families, and researchers, funded by the SMA Foundation. A major aim is to explain how emerging therapies attempt to "turn up" production of an essential muscle protein by correcting or compensating for an error in RNA splicing. Interviews with patients and clinicians are complemented by interviews with researchers, including CSHL alumni Philip Sharp and Richard Roberts, who shared the Nobel Prize for the discovery of RNA splicing. Animations illustrate the physiological and molecular genetic basis of SMA and emerging therapies. A detailed three-dimensional animation of the mechanism of RNA splicing was produced by Drew Berry, a 2010 McArthur Fellow with whom we collaborated on the award-winning *DNA Interactive* website and the PBS television series "DNA."

As outreach for an NSF research project on maize growth, with CSHL researcher Marja Timmermans and Cornell researcher Mike Scanlon, we completed development of *Weed to Wonder*. This "e-book" tells the story of how human ingenuity transformed a common Mexican weed (teosinte) into a modern food wonder (maize). Using state-of-the-art software, we created a flexible, *National Geographic*-style magazine that can be ported as an Internet microsite, tablet-enabled application, or printable PDF. The tablet application shows off the real power of the technology, where pages advance by "swiping" and videos automatically expand to play full screen. The work takes readers on a journey from the domestication of maize in Mexico to the development of modern hybrids, genome sequencing, transposons, and direct genetic modification and biofortification. The history of maize research is brought to life through reconstructions of George Shull's and Barbara McClintock's work at Cold Spring Harbor Laboratory, rare photographs, and links to original publications and artifacts.

As part of the project, we filmed a time-lapse video of the life cycle of maize plants—from emerging cotyledons to harvest, during a period of 105 days. With help from Peter Bommert, Tim Mulligan, and the CSHL facilities staff, we installed a minigarden of six plants on the DNALC roof and automatically recorded numerous clips per day on two video cameras installed behind a Plexiglas screen. The plants weathered the early onset of Hurricane Irene, but they were brought inside for the worst of it. Once the hurricane was past, video capture continued using battery packs for several days until power was restored to the DNALC.

Urban Barcode Project

The *Urban Barcode Project* (*UBP*) is a science competition to support independent, open-ended investigations using DNA barcoding by New York City (NYC) high school students. Supported by an 18-month grant from the Alfred P. Sloan Foundation, we aim to show that we can quickly develop an infrastructure to support large-scale dissemination of student DNA barcoding in NYC and other sites around the world as outreach for the *International Barcode of Life Project* (iBOL).

In 2011, we completed development of a robust protocol to extract, amplify, and sequence DNA barcodes from many plant, fungal, and animal species. The experiment is supported by a dedicated Internet microsite, www.DNAbarcoding101.org, which includes online and downloadable lab protocols, detailed teacher preparation materials, video podcasts ("vodcasts"), animations, and a news feed. In 2012, the experiment will be published in the DNALC's forthcoming lab/text *Genome Science* (by CSHL Press) and as a stand-alone kit marketed by the Carolina Biological Supply Company. DNA sequencing is provided at $3.00 per read by GENEWIZ, with results automatically uploaded to *DNA Subway*.

In addition to this research infrastructure, we also built a *UBP* website to support all aspects of the project (www.urbanbarcodeproject.org), including vodcasts on barcoding and student projects, guidelines for proposal preparation, and management tools for tracking student projects from submission to completion. During the first 6 months of the year, we engaged in a blitz of promotion, with targeted e-mails to 10,500 NYC teachers and administrators, superintendents, principals, and teachers in NYC public and private high schools, 21,500 e-copies of the DNALC's *BioBulletin*, and posters and brochures delivered to 150 high schools and 25 universities. Nearly 800 high school teachers, administrators, and researchers attended 19 information sessions, and 128 teachers and mentors attended 18 workshops for training on the DNA barcoding experiment. These 6-hour workshops dovetailed with our existing Howard Hughes Medical Institute (HHMI) teacher-training program.

Our advertising, recruitment, and training strategies worked. After careful review, we accepted 102 of 118 project proposals, meeting our goal of involving 100 teams in the first year of the project. The teams comprise 304 students (including 26% Latino or African American) from 31 public and nine

private schools across the five boroughs of NYC. Teams are mentored by 36 high school teachers, four graduate students, two postdoctoral fellows, and six university professors. Projects examine biodiversity in parks and public areas (42%), species traded in ethnic markets (28%), food fraud (17%), exotic and invasive species (8%), and public health and disease vectors (5%). To assist with collecting specimens, we coordinated permits and activities with the New York State (NYS) Department of Environmental Conservation and Department of Parks and Recreation. Participating teams have free access to equipment and reagents needed to isolate DNA and amplify the barcode region from their samples. They may check out an equipment footlocker for use at their own school or attend *Open Lab* sessions at *Harlem DNA Lab* and Genspace, a nonprofit citizen science center in Brooklyn. All students have access to a DNALC staff member or trained mentor. Teams will present their study results in poster sessions held in spring 2012. A jury of experts in biodiversity, conservation biology, DNA barcoding technology, and science education will select the top teams based on project originality, creativity, relevance, thoroughness, and scientific merit. Finalists will give oral presentations at a symposium, where teams winning the $10,000 grand prize and $10,000 in runner-up prizes will be announced.

iPlant Collaborative

The most obvious driver of plant research is the race to develop higher-yielding plants to feed a burgeoning world population. Aside from more human mouths to feed—world population hit 7 billion in October 2011—there are also more livestock to feed. Increasing wealth in developing countries means that more people can afford Western diets where more calories come from meat raised on grain. Global climate change adds new urgency to this age-old problem, and genome scientists are joining forces with plant breeders to help crop plants adapt to regional changes in rainfall and temperature.

The *iPlant Collaborative* is a major NSF project to develop a computer infrastructure to help plant scientists increase global food production in the face of climate change. A consortium of the University of Arizona, University of Texas at Austin, and CSHL, *iPlant* focuses on building computer tools and services to solve two "Grand Challenges" in plant research: *Genotype to Phenotype* aims to predict how genes and the environment work together to influence plant traits, and *Tree of Life* works to build a phylogenetic tree that represents the evolutionary relationships of all species of plants. Both efforts are made possible by new technologies, such as next-generation DNA sequencing, which can produce an amount of data equivalent to the human genome in less than a week.

During the year, *iPlant* launched three major legs of its cyberinfrastructure: (1) *Discovery Environment* is a customizable workspace for a variety of data analyses, (2) *Data Store* is a secure place to make large-scale user data available to *iPlant* analysis tools, and (3) *Atmosphere* is a personal, customizable computing cloud to run analyses and share projects. With the maturation of the *iPlant* cyberinfrastructure, education, outreach, and training (EOT) is becoming a primary concern of all *iPlant* staff and collaborators.

As leader of the EOT effort, the DNALC lends its expertise in organizing workshops, developing training materials, and building websites and interfaces. During the first half of the year, the DNALC's *BioMedia* Group redesigned the *iPlant* website to streamline navigation and better convey the project's goals and progress. Vodcasts provide overviews and guided tutorials to acquaint scientists with *iPlant*'s many tools and services. The improved website was relaunched in July with remarkable results: Visits increased 64% July–December 2011 (21,881) compared to the same period in 2010 (13,331).

We continued to evolve *DNA Subway* as the educational analog of the *Discovery Environment. DNA Subway* is an intuitive bioinformatics platform based on the metaphor of a subway line. Most effort focused on development of the Blue Line for DNA barcode and phylogenetics analyses. The Blue Line includes a number of new web applications that heretofore could only be purchased as stand-alone desktop software. An electropherogram viewer allows users to visualize the peaks that determine DNA sequence "reads," and a DNA barcode viewer makes it easy to see patterns of single-nucleotide polymorphisms (SNPs) between aligned DNA sequences. A consensus builder and editor helps users maximize data from forward and reverse reads. Automatic links to Google images, Wikipedia, and the Encyclopedia of Life provide context to DNA sequence search (BLAST) results. Phylogenetic trees are constructed using both neighboring joining and maximum likelihood methods. Toward the end of 2011, the team began working with the NIH National Center for Biotechnology Information (NCBI) to develop a pipeline for users to submit novel barcode sequences to GenBank.

DNALC staff introduced *DNA Subway* and barcode analysis to more than 300 high school and college educators at workshops conducted at nine locations across the United States. An additional 222 educators learned about *DNA Subway* at short courses and demonstrations at meetings of the American Society of Plant Biologists (ASPB), Botanical Society of America, National Association of Biology Teachers (NABT), National Science Teachers Association (NSTA), *iPlant Collaborative*, and USDA Plant and Animal Genome Research Program.

Howard Hughes Medical Institute

Virtually all students in NYC receive instruction in genetics and biotechnology at two key points in their education: in 8th grade as part of the NYC *Scope and Sequence for Science* and in 9th–10th grade in the NYS Regents course, *Living Environment*. Many teachers in the middle grades do not have sufficient training and have very few resources for providing hands-on, inquiry-based labs to make genetics and biotechnology content interesting and relevant for students. Thus, the objective of the HHMI project is to train teachers to use a set of six targeted labs that cover key concepts and techniques within the NYC and NYS curricula: variability and inheritance, DNA structure and isolation, bacterial transformation, protein isolation, DNA analysis and forensics, and analysis of human DNA variations by polymerase chain reaction (PCR).

In 2011, 146 science teachers attended HHMI workshops at the *Harlem DNA Lab:* 128 teachers participated in 44 1-day workshops, and an additional 18 highly qualified teachers participated in the 2-week *Leadership Symposium*. More than three-quarters of the trainees were within our primary audience of 8th–10th-grade teachers. Notably, nearly half of the workshop participants were African American or Hispanic (48%), although these groups comprise only 14% of teachers nationwide (U.S. Census Bureau, 2011 Statistical Abstract).

Evaluating pre- and postworkshop surveys of 2011 participants revealed significant knowledge gains for all workshops (pretest mean score 74% ± 16 vs. posttest 81% ± 14; $t_{56} = 5.535$, $P < 0.001$). Follow-up surveys showed that of the 2009 and 2010 program participants, 81% had improved

confidence in teaching labs, and a majority had implemented at least one of the targeted labs (59%) or used the DNALC's online teaching resources (61%) during the last 12 months.

Support of in-class instruction became a crucial focus in 2011. Through a grant from the Richard Lounsbery Foundation, we developed 15 biotechnology footlockers that contain all of the equipment and expendable supplies needed to teach each targeted lab. It took us a little time to figure out why footlocker use was slow in 2010; logistically, there was no way for teachers to get the bulky kits to their schools by public transport and many could not afford the modest restocking fee. Thus, we now use unrestricted funds from the Dana Foundation to pay for restocking and FedEx shipping. A DNALC instructor is also available to help set up the equipment and co-teach the lab activities in school. As a result, footlocker use doubled in 2011, with 2567 students participating in experiments at their schools, 60% of whom were African American or Hispanic.

HHMI program participants are supported by dedicated online resources for each targeted lab. Each *Lab Center* is a virtual classroom set, into which a DNALC staff member enters to make a video introduction. By clicking on various objects on the whiteboard and desk, users can access (1) interactive and PDF versions of the experimental protocol, (2) follow-up activities, (3) scientist interviews, (4) animations, and (5) selected links to related Internet resources. *Lab Center* content was developed and reviewed by six HHMI Teacher Fellows, who are seasoned educators from the New York metropolitan area.

In 2011, we developed the pilot *New York Stories*, a series of vodcasts to highlight research done at New York institutions related to each of the target labs. The pilot production was led by HHMI Teacher Fellow Kathleen Rucker and science teacher David Conneely of Brooklyn International High School. A six-student production team developed a rough script/storyline for bacterial transformation. In parallel with a *BioMedia* videographer, the students filmed themselves with Flip cameras as they performed a transformation experiment at school and then traveled to Columbia University to interview Nobel laureate Dr. Martin Chalfie. Stop-motion animations and intercutting low- and high-resolution footage contributed to an edgy production with an MTV feel.

New National Science Foundation Training Program

Careers in biotechnology increasingly demand a genome-wide perspective that requires students to move comfortably between in vitro experiments at the lab bench and in silico experiments on the computer. In the spring, the DNALC was awarded a grant from the NSF Advanced Technological Education (ATE) Program to help community college faculty move students toward this synthesis. In collaboration with the National Advanced Technological Education Center for Biotechnology (Bio-Link), we presented *Genomic Approaches in Biosciences* workshops that provided the scientific foundation for biotechnology careers in the genome age. The program focuses on four key technologies—PCR, DNA sequencing, RNA interference (RNAi), and bioinformatics—in three eukaryotic systems: humans, plants, and *Caenorhabditis elegans* (roundworm). Each experiment/bioinformatics module can be integrated into existing courses, provide the basis for new courses, or serve as the foundation for student research projects.

During its 3-year term, the program will reach 288 biotechnology faculty at week-long workshops conducted at 12 community colleges nationwide. In spring, we convened a board of 12 faculty advisors who are involved in all aspects of the program: determining the workshop curriculum, hosting and co-instructing workshops, providing follow-up participant support, and evaluating the program. In the summer, 88 educators participated in workshops conducted in four locations: Gwinnett Technical College (Lawrenceville, Georgia), Madison Area Technical College (Madison, Wisconsin), Shoreline Community College (Shoreline, Washington), and Universidad del Turabo (Gurabo, Puerto Rico). Postworkshop surveys of participants showed gains in concept knowledge and teaching confidence, particularly for DNA barcoding, RNAi, and bioinformatics, and 82% said they would implement the materials in their teaching.

The ATE program makes use of two DNA sequencing projects developed by the DNALC. DNA barcoding, developed for the *iPlant Collaborative* and the *Urban Barcode Project*, is described above. Human mitochondrial DNA sequencing, developed in 1998, allows students to examine a variable DNA sequence from their own genome. Using a kit developed with our collaborator, Carolina Biological Supply Company, students isolate DNA from cheek cells, amplify part of the mitochondrial control region, and send their samples for DNA sequencing. With kits donated by Applied Biosystems, the DNALC provided free DNA sequencing from 1998 through 2010.

Anticipating increased demand for sequencing from three grant-supported programs, we calculated that the cost of commercial sequencing had declined so much by 2011 that it was less than our costs to have a college intern do the lab work! Thus, we contracted with GENEWIZ to provide DNA sequences for the DNALC and affiliated schools for $3.00 each. This had the effect of improving sequence quality and reducing turnaround time from 2 weeks to 2 days. Under this new paradigm, a record number of sequences were uploaded to DNALC databases. A total of 8436 student samples (7073 mitochondrial control region sequences and 1363 DNA barcodes) were submitted from 110 high schools and 67 colleges and universities.

Nationwide Teacher-Training Programs

For more than 25 years, the DNALC has provided cutting-edge lab and computer training for educators at sites around the world. In 2011, 457 high school and college faculty participated in workshops conducted at 19 sites across the United States. These were in addition to the 273 NYC teachers trained with support from HHMI and the Sloan Foundation, where 40% of participants were minorities underrepresented in science (African Americans and Hispanics). Overall, underrepresented minorities composed 27% of 730 educators trained at 76 workshops (1–10 days). An additional 1400 people attended seminars or short workshops at professional meetings, including ASPB, iBOL, NABT, NSTA, International *C. elegans* Meeting, and Plant and Animal Genomes. (For a complete list of training activities and host sites, see the table at the back of this report, "2011 Workshops, Meetings, and Collaborations.")

In addition to the student experimental studies highlighted in the introduction, we were interested in learning the effects of three teacher-training programs that concluded in 2011: *Silencing Genomes, Genes to Cognition (G2C) Online*, and *Inside Cancer*. In each case, faculty were surveyed at three time points: preworkshop, postworkshop, and 9–18 months later. This added to more than 20 years of longitudinal DNALC studies tracking the effects of workshop training on classroom teaching behaviors.

Silencing Genomes, funded by the NSF Course Curriculum and Laboratory Improvement (CCLI) Program, introduced faculty to the Nobel-Prize-winning technology of RNAi. The 5-day workshops clearly had their intended effect of increasing hands-on lab experience. Before the workshop, only 6% of participants had done labs, but 12–18 months later, 71% had done at least one lab. Remarkably, 20% of educators had students develop custom RNAi vectors, and 43% had students do complementary bioinformatics exercises. Respondents reported doing the new labs with 3926 students and providing classroom lectures on RNAi to 10,053. There was also a marked "train-the-trainers" effect, with workshop participants providing labs to 156 other instructors and lectures to 392 faculty. To support classroom implementation, the DNALC built a collection of *C. elegans* strains and *Escherichia coli* RNAi feeding strains, which are available free of charge through the *Silencing Genomes* website. A formatted e-mail allows educators to request strains for specific dates. Since launching the service in August 2007, we have filled orders for more than 2800 strains, which educators reported using with more than 16,000 students.

Likewise, the majority of participants in 1-day workshops on *G2C Online* and *Inside Cancer* reported improved understanding and use of the multimedia materials to scaffold instruction in a variety of classes.

	G2C Online	Inside Cancer
Reported good to very great improvement in		
Understanding of topic	88%	54%
How to teach the topic	90%	61%
Had used website with one or more classes since workshop	86%	97%
Classes in which materials were implemented		
General biology	42%	50%
Honors/AP biology	19%	33%
Genetics/Biotechnology	14%	27%
Anatomy and physiology	14%	20%
General/AP psychology	19%	N/A
How website was used		
As a source of information	70%	82%
As a source of new teaching materials	63%	64%
To read scientific articles	52%	55%
For student projects	48%	37%
To teach directly in the classroom	39%	45%

Dolan and DNALC *West* Student Programs

This year, we served 25,836 student participants in both in-school instruction programs and field trips to the Dolan and *West* facilities. Participation of 2800 students from underserved Long Island and Queens school districts was sponsored by grants from Bank of America and the National Grid Foundation. This included our continuing collaboration with Brentwood Union Free School District, where we provided genetic engineering labs for 6th–8th grade classes participating in the first district-wide science fair. The entire world was inspired when Samantha Garvey, a former participant in DNALC labs at Brentwood, overcame homelessness to become a semifinalist in the Intel Science Competition.

The summer workshop season was record-breaking in every sense. Topping the previous record from 2009, more than 1000 students participated in 44 week-long camps held at six locations on Long Island and NYC: Dolan DNALC, DNALC *West*, Stony Brook University, Chapin School, and Trinity School. Among the summer campers were 19 students from Beijing No. 166 High School, the first specialized school of life sciences in the Chinese capital. The students spent three weeks at the DNALC, progressing from classical genetics, to bacterial cloning, to human and plant genomics. The summer study culminated in a book signing and Q&A session with CSHL's own Nobel laureate, James Watson, and a field trip to the Human Origins exhibit at the American Museum of Natural History. Anticipating a growing exchange program with Beijing 166 and other schools in China, we proceeded with plans to develop a fourth teaching lab at the Dolan DNALC.

Students, teachers, and administrators from Beijing No. 166 pose with DNALC staff.

We struggled to save the last week of summer camps in the aftermath of a visit by Hurricane Irene, which took out power for more than 350,000 homes and businesses on Long Island. The Monday following the storm, we returned to find power off, the basement flooded, and more than 50 families arriving with camp registrants. After meeting parents at the curb and communicating a

hurried plan, we spent the rest of the day moving three labs' worth of equipment and supplies to teaching spaces on the CSHL campus. By Tuesday morning, all was in place to restart the camps, with greeters directing parents to locations in Beckman and Delbrück labs, and 95% of registered campers turning up.

Monthly *Saturday DNA!* sessions drew 219 participants, with parents and grandparents joining their children to learn about superbugs, dinosaurs, and mitochondrial DNA. The annual *Great Moments in DNA Science* seminar series drew 162 top Long Island high school students for presentations on current biological research. CSHL researcher Dr. Andrea Eveland explained how she combines classical genetics with cutting-edge technologies to understand the molecular mechanisms that control flowering in maize. Dr. Damon Love, a postdoctoral researcher at Weill Cornell Medical College, discussed how RNAi can potentially silence genes involved in the progression of liver cancer. Dr. Benoit Boivin from the Tonks lab at CSHL explained how cell physiology is affected when oxygen reacts with a class of enzymes called protein tyrosine phosphatases.

Harlem DNA Lab

With continued core support from the Dana Foundation and student scholarship funding from the William Townsend Porter Foundation, in 2011 the *Harlem DNA Lab* hosted 1849 NYC public and private school students, 60% of whom were either African American or Hispanic. We also provided intensive enrichment to students from two schools housed with us in the John S. Roberts (JSR) Educational Complex: 12th graders from the Coalition School for Social Justice participated in three biotechnology laboratories, and 6th–8th graders from MS45 enjoyed three introductory genetics laboratories. In the spring, we participated in the World Science Festival, hosting children at an outdoor DNA extraction booth in Washington Square Park. *Harlem DNA Lab* also became the focal point for our *Urban Barcode Project*, hosting information and training sessions for educators and providing kits and *Open Lab* days for teams to conduct their experiments.

Visitors to our booth at the World Science Festival view cells and extract DNA.

The Charter Membership Program continued in 2011 with Chapin School and Trinity School. The program provides intensive support to independent schools wishing to develop a sequenced program of accelerated science opportunities for students and teachers. Each school receives customized instruction that includes professional development for science teachers, field trips to DNALC facilities, in-school lab visits, and on-site summer camps taught by DNALC instructors. As a result of this successful partnership, we worked with Chapin School's Head of School, Patricia Hayot, to develop an extensive program of DNA camps for students from NYC independent schools to be held at Chapin in summer 2012.

Although we have managed to stay afloat against a current of change within the NYC public school system, the continued devolvement of the Department of Education (DOE) is a source of unease. The JSR Educational Complex is emblematic of continued reorganization and administrative shuffling within the DOE, with two schools phased out and two new schools phased in during our tenancy. Student discipline and security are recurring concerns. HHMI support has helped us prove that a small, focused science institution can indeed have a substantial impact on a megalithic school system. However, at this point, it seems imperative that we establish a standalone facility in Manhattan, where we can continue to expand teacher and student opportunities to participate in the gene age.

Partnerships and Graduate Training

We continued our partnership with Cold Spring Harbor High School (CSHHS) to offer *Molecular and Genomic Biology*. This college-level course is scheduled during the last two class periods and is co-instructed by DNALC staff members and CSHHS biology and research teacher Jaak Raudsepp. The course emphasizes hands-on experimentation, critical thinking, and independent projects across a range of biological systems. The sixth class started in the fall with a unit on gene function in the roundworm *C. elegans*. CSHL Watson School of Biological Sciences (WSBS) graduate student Colleen Carlston helped the CSHHS students set up a genetic screen that identified three new genes that affect RNAi function. The class then moved on to projects using DNA barcoding to identify wild plants and animals, pets, and food products from grocery stores.

A project on three-dimensional protein modeling was conducted in collaboration with the Center for Science and Mathematics Education (CESAME) at Stony Brook University. The students used data from primary research papers to identify important parts of proteins involved in the cell cycle and drugs that interact with them. They then used three-dimensional modeling software to highlight key atoms involved in molecular interactions. The modified structure files were sent to CESAME, where detailed atomic resolution models of the proteins were manufactured on a state-of-the-art prototyping three-dimensional printer. This machine is essentially a laser printer that layers droplets of polymer resin in three dimensions to build a detailed molecular model. Students will present their models and explanatory posters at the May 2012 CSHL meeting, *The Cell Cycle*. (A highlight of last year's course was students' poster presentations at the CSHL *Biology of Cancer* meeting, where the audience included Nobel laureate Robert Weinberg.)

As part of our expanding collaboration with CESAME, Multimedia and Evaluation Manager Amy Nisselle, Ph.D., was appointed an adjunct faculty member at Stony Brook University. Calling on her academic background in public health, education, and medical research, Amy coadvises CESAME doctoral program candidates on thesis projects. Two candidates are helping to evaluate the NSF ATE *Genomic Approaches in Biosciences* program: DNALC Teacher Fellow Caren Gough is investigating the program's impact on teachers' pedagogical beliefs and practices, and Robin Tornabene is examining its socioscientific effects. Jaak Raudsepp of CSHHS is tracking the long-term career trajectories of CSHL *Partners for the Future* alumni.

In our collaboration with the WSBS, graduate students engage in a 3-month rotation in science instruction. Pairs of students work with a seasoned DNALC instructor to master the presentation of genetics labs to middle and high school students. During the first phase of training, student teams observe the DNALC instructor in the classroom and organize a lesson plan that integrates their own perspectives. The second phase is coteaching, during which graduate students instruct the lab portion of the class. DNALC instructors provide constructive critique in preparation of the third and final phase: independent teaching. After completion of both middle and high school teaching rotations, WSBS students deliver three additional lessons to demonstrate mastery of instruction and classroom management skills, which are useful for teaching at any level.

We applied our expertise in graduate education to a collaboration with the New York Academy of Sciences (NYAS). One of the oldest scientific institutions in the United States, the NYAS is now based in the new World Trade Center. The DNALC provided 6 hours of lab instructional training to 100 graduate students and teachers who serve as NYAS mentors in after-school programs for NYC students in grades K–12. The mentors learned to deliver several popular DNALC labs, including the *DNA Extraction from Banana* and *Baggie Cell Model*.

We also continued our long-term collaboration with the Singapore Ministry of Education, hosting six Singaporean teachers for 2-week attachments. In July, high school biology teachers Ms. Leo Minyin and Mr. Yang Wenjun participated in HHMI Leadership training at the *Harlem DNA Lab*. In November, primary teachers Mrs. Thia Woon Ling, Ms. Eileen Chin, Mdm. Wee Shu Yi, and Mr. Azman bin Mohammed Dali participated in student labs and small workshop sessions focused

on *Fun with DNA* and *World of Enzymes* curricula. All teachers are required to author formal lesson plans and share new teaching methods with teachers at their home schools.

Staff and Interns

The year 2011 saw a number of significant staff changes, with bittersweet partings and promises of new challenges. Uwe Hilgert wound up 10 years' service to the DNALC and CSHL when he returned to the University of Arizona to become director of outreach for the Bio5 Institute. Reared on a riverboat in Germany, Uwe received a Ph.D. from the Max Planck Institute for Plant Breeding in Cologne and then conducted postdoctoral research at the University of Arizona. Uwe started at the DNALC in 2000 as a high school instructor, then quickly developed the DNALC's bioinformatics capability under an HHMI grant. As Assistant Director, beginning in 2005, Uwe assumed responsibility for all of the DNALC's professional development activities, which each year involved planning several dozen events around the country. After a year's special assignment as Assistant Dean of the WSBS, where he helped ease transition to new leadership, Uwe returned to the DNALC in 2008 to spearhead the outreach component of the *iPlant Collaborative*. His attention to detail and ability to carry out complex planning was critical during a period when DNALC's teacher training increased fivefold. Although he will be greatly missed, we continue to collaborate on a weekly basis.


Uwe Hilgert and Sheldon McKay

Ileana Rios left her position as *Harlem DNA Lab* manager for a teaching position at the prestigious Trinity School, where her son is a student. Ileana was the perfect person to initiate our effort to bring high-level science to underrepresented students in NYC. She was born and attended elementary school only blocks away from our facility in Harlem, received a bachelor's degree in biology from Barnard College and Ph.D. in molecular biology from City University of New York (CUNY). Her background and work at *Harlem DNA Lab* will come in handy as she develops a molecular genetics elective at Trinity and as several teams of her students participate in the *Urban Barcode Project*.

Under normal circumstances, the loss of two key staff would have been traumatic, but we were lucky to have two fantastic replacements waiting in the wings. Uwe's loss was compensated by a reciprocal move back to CSHL by Sheldon McKay, who had moved from Lincoln Stein's lab to the *iPlant Collaborative* at the University of Arizona. Sheldon brings high-level bioinformatics expertise to the DNALC. Sheldon has bachelor's and master's degrees in genetics from the University of British Columbia and a Ph.D. in evolutionary genetics from Simon Fraser University. His first task will be to build Green Line for our *DNA Subway*; this workflow will provide a simple interface to analyze RNA data sets from next-generation sequencers.

New *Harlem DNA Lab* Manager Melissa Lee has an undergraduate degree in biology from Johns Hopkins and a master's degree in science education from CUNY. Her work experience includes managing a molecular genetics lab at New York University and, for the last several years, teaching at Marie Curie Science High School in the Bronx. As a student growing up in the Bronx, Melissa's interest in genetics was sparked when she attended a *DNA Science* course taught by the DNALC at the AMNH.

At the same time, we recruited Oscar Pineda-Catalan as *Urban Barcode Project* Manager. A native of Mexico City, Oscar has an undergraduate degree in biomedical basic research and a master's degree in urban studies from the University of Mexico. Teaching biology, human anatomy, and physiology in a Mexico City high school inspired him to pursue a career encompassing both scientific research and education. Under a Fulbright Fellowship, he completed a Ph.D. in ecology and conservation biology at Columbia University, where his thesis examined threatened turtles of the

Amazon River in Ecuador. George Amato graciously allowed him to keep an office at the Sackler Institute for Comparative Genomics at the AMNH, where he most recently did postdoctoral research. This significantly strengthened our collaboration with this important institution.

Oscar Pineda-Catalan, Melissa Lee, Alexandra Manaia, and Christine Marizzi

Our outreach effort was further bolstered with two incredible staff members from Europe. A native of Austria, Dr. Christine Marizzi divides her time between the *Harlem DNA Lab* and DNALC *West*, where she is the new Lab Manager. After receiving a Ph.D. in genetics from the University of Vienna, Christine developed youth education programs with *wienXtra* and was an instructor at *Vienna Open Lab*, a long-term licensee of DNALC intellectual property. Dr. Alexandra Manaia is working on the *UBP* in fulfillment of the final phase of a Fulbright Fellowship and master's degree in international education development at Columbia University. After receiving a Ph.D. in developmental biology from Paris 7 University, she worked as science education officer at the European Molecular Biological Laboratory in Heidelberg, where Dave Micklos met her when working as an advisor to a Europe-wide teaching training program.

The DNALC bid farewell to middle school instructor Jennifer Aiello in 2011. Jen started at the DNALC as a high school intern in 2002 and worked her way up to instructor after completing a bachelor's degree in forensic science in 2009 from Long Island University. In November, Jen accepted a position with the NYC Police Department to pursue a career in criminalistics and forensics. We also said goodbye to videographer Todd Rocheford, who will use his experience developing *New York Stories* and *UBP* videos in his new consultancy.

Our internship program continued to draw some of Long Island's most talented high school and college students, engaging them in science research and providing practical laboratory experience. Our new high school interns were Kathryn Bellissimo (Walt Whitman), Cyril Danielkutty (Harborfields), Alexa DeAngelis (St. Anthony's), Frieda Haerter, Julie Hemphill, Eun Sung Suh (John Glenn), Paras Patel (Half Hollow Hills East), Anna Saum (home schooled), and Gianna Torre (Wantagh). The new hires joined a dedicated group of returning interns: Jack Greenfield (Oyster Bay), Devika Gupta (Farmingdale), Jueng Woen Kim (Hauppauge), and Young Joon Suh (John Glenn). During the summer, we welcomed four new interns to assist with DNALC *West* camps: Eliana and Juliet Jacobson (Hebrew Academy of Nassau County), James Polke (Regis), and Aman Sharma (Herricks). We bid farewell to a number of high school interns as they left for their freshman year at college: Laura Bergsten (Dartmouth), George Economou, Lindsay Hochberg, David Streitman (Cornell), Max Vaysman (SUNY Geneseo), and Pamela Wax (Northwestern).

College interns supervise high school students, assist with summer camps, and work on specialized projects, including fulfilling faculty requests for RNAi targeting vectors. Returning college interns in 2011 were David Dopfel, Lina-Mari Varghese (Stony Brook University), Katherine Villalon (John Jay University), and Sara Weinclaw (University of Maryland). City-dwellers Arielle Scardino (City College of New York) and Sulaiman Usman (New York Institute of Technology) assist at *Harlem DNA Lab* and with the *UBP*.

Many DNALC interns are involved in independent research projects and compete in state and national science competitions: Alexa DeAngelis studied visual and spatial short-term memory loss, Young Joon Suh studied bioremediation of oil spills using cyanobacteria, Eun Sung Suh studied effects of antibiotics on *C. elegans*, Katherine Villalon used random amplified polymorphic DNA (RAPD) to detect genomic instability in lung cancer, and David Dopfel examined longevity genes in *C. elegans*.

We also welcomed two *BioMedia* college interns in 2011. Mara Smith is completing a degree in neuroscience and science journalism at Brown University. Under a Royce Fellowship, she assisted

with the development of the *Learn About SMA* Internet microsite. Anne Burlet-Parendel returned to CSHL in 2011 after studying neural stem cells in the Grisha Lab as part of her master's in genetics, University Denis Diderot, Paris, in 2010. In preparation for starting a master's in science communication at the University Louis Pasteur in Strasbourg, Anne assisted with several projects and inventoried DNALC lab instructional assets.

Expert Advisors and Corporate Support

The DNALC benefits from a Corporate Advisory Board (CAB) that focuses on fundraising from the Long Island business community. Under the guidance of development staff member Karen Orzel since 2005, the CAB golf outing and annual fund have contributed more than $200,000 per year to DNALC operations. Also during that time, CAB members Laurie Landau, Eddie Chernoff, Peter and Dori Tilles, Pall Corporation, and OSI Pharmaceuticals provided major endowment gifts.

Over the years, the CAB has operated in parallel with the CSHL Association. This group of "friends of the Lab" has raised annual funds for CSHL since the 1920s. To unify local fundraising and foster a greater sense of common purpose, the CAB and CSHL Association annual funds were merged in 2011. Although the two organizations will continue to be managed by separate boards, the unrestricted funds they raise will support both the research and education missions of CSHL.

2011 Workshops, Meetings, and Collaborations

January 15	HHMI Professional Development, *Detecting Genetically Modified Organisms* Workshop, *Harlem DNA Lab*
January 15–19	Plant and Animal Genome XIX Conference, Town and Country Convention Center, "*DNA Subway:* The Fast Track to Gene Annotation," San Diego, California
January 21–22	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, Florida A&M University, Tallahassee, Florida
January 22	Graduate Student Training for STEM Mentoring Program, New York Academy of Science, New York
	Saturday DNA! "Mitochondrial DNA—Mom's Legacy," DNALC
January 26–28	NSF 2011 Course, Curriculum, and Laboratory Improvement—Transforming Undergraduate Education in Science (CCLI-TUES) Conference, Washington, D.C.
January 29	Graduate Student Training for STEM Mentoring Program, New York Academy of Science, New York
February 4	Site visit by Christine Marizzi, Max F. Perutz Laboratories, Vienna, Austria
	HHMI Professional Development, *DNA Structure and Isolation* Workshop, *Harlem DNA Lab*
February 5	HHMI Professional Development, *DNA Structure and Isolation* Workshop, *Harlem DNA Lab*
February 8	Site visit by Wang Lei, Zhu Yan, and Li Zhenghua, Beijing No. 166 High School, Beijing, China
February 12	HHMI Professional Development *Discovering the Urban Environment* Workshop, New York Academy of Science, New York
February 18–20	Science Without Borders, The American Association for the Advancement of Science Meeting, "Search Engine Optimization to Increase Audiences for Online Science Education," Washington, D.C.
February 25–26	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, University of Arizona, Tucson
February 26	*Saturday DNA!* "Dino DNA!," DNALC
	HHMI Professional Development, *Variability and Inheritance* Workshop, *Harlem DNA Lab*
March 8	Site visit by Father James Williams and John Westerman, Chaminade High School, Mineola, New York
March 8–9	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, City College of San Francisco, California
March 11–12	National Science Teachers Association 2011 National Conference on Science Education, "Barcoding Plants," "Frontiers in Genomics," "RNAi/CBSC," "DNA Subway," "Iceman," "Lab Center," and "CSI," San Francisco, California
March 12	HHMI Professional Development, *DNA Transformation and Protein Isolation* Workshop, *Harlem DNA Lab*
	Urban Barcode Project Information Session, New York Academy of Sciences, New York
March 19	*Urban Barcode Project* Information Session, *Harlem DNA Lab*
March 21–24	Joint Genome Institute User Meeting, Walnut Creek, California
March 25	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
March 26	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
	Saturday DNA! "Recovering the Romanovs," DNALC
	Urban Barcode Project Information Session, The Rockefeller University, New York
April 2	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
	Urban Barcode Project Information Session, SCONYC Conference, Stuyvesant High School, New York
April 8	HHMI Professional Development, *PCR and Human DNA Variation, Part 1* Workshop, *Harlem DNA Lab*
April 9	HHMI Professional Development, *PCR and Human DNA Variation, Part 1* Workshop, *Harlem DNA Lab*
	Urban Barcode Project Information Session, American Museum of Natural History, New York
April 15–16	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, North Carolina A&T State University, Greensboro
April 16	*Saturday DNA!* "Milky Menace," DNALC
April 27	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
April 30	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
April 30–May 3	NSF ATE *Genomic Approaches in Biosciences*, Advisory Board Meeting, CSHL
May 5	*Great Moments in DNA Science* Honors Seminar: "Sowing the Next-Generation 'Green Revolution': Genetics and Genomics of Maize Development," Andrea Eveland, CSHL, DNALC
May 6	HHMI Professional Development, *PCR and Human DNA Variation, Part 2* Workshop, *Harlem DNA Lab*
May 6–7	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, Bowie State University, Maryland
May 7	HHMI Professional Development, *PCR and Human DNA Variation, Part 2* Workshop, *Harlem DNA Lab*
May 8–12	National Institutes of Health Science Education Partnership Award Principal Investigators Conference, "Nationwide Dissemination of *Inside Cancer*," Seattle, Washington
May 10	Site visit by Leo Brizuela and Jim Lynch, Agilent, Santa Clara, California
	Great Moments in DNA Science Honors Seminar, "A Gene Silencing Approach to Control the Development of Liver Cancer Cells," Damon Love, Weill Cornell College of Medicine, New York, DNALC
May 12	Site visit by Leo Brizuela, Agilent, Santa Clara, California
	Urban Barcode Project Information Session, New York Academy of Sciences, New York

May 14	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
May 18	Science Coordinators Network Meeting, Dowling College, Oakdale, New York
May 21	*Saturday DNA!* "Personalized Medicine," DNALC
May 26	*Great Moments in DNA Science* Honors Seminar, "How Much Is too Much? Tracking Down Oxygen-Dependent Modifications in Normal and Diseased Cells," Benoit Boivin, CSHL, DNALC
June 4	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
June 4–5	World Science Festival, "*Harlem DNA Lab*," and "*DNA Subway*," New York
June 6–10	Teacher Professional Development Workshop, The Chapin School, New York
June 7	18th Annual Golf Outing, Piping Rock Club, Locust Valley, New York
June 10	NSF Shoot Apical Meristem Meeting, CSHL
June 10–11	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, Canisius College, Buffalo, New York
June 11	*Urban Barcode Project* Training Session, Genspace, Brooklyn, New York
June 13–17	*DNA Science* Workshop, Trinity School, New York
	Fun with DNA Workshop, The Chapin School, New York
	Fun with DNA Workshop, Trinity School, New York
	Human Genomics Workshop, The Chapin School, New York
	World of Enzymes Workshop, The Chapin School, New York
June 16	Site visit by Gregory Crawford, University of Notre Dame, Indiana
June 18	*Saturday DNA!* "Express Yourself!" DNALC
June 20–24	NSF ATE Professional Development, *Genomic Approaches in Biosciences* Workshop, Gwinnett Technical College, Lawrenceville, Georgia
June 22–26	18th International *C. elegans* Meeting, University of California, Los Angeles
June 27–July 1	World Conference on Educational Multimedia, "Novel Networked Navigation: A Case Study of a Genetics Education Internet Site," Lisbon, Portugal
	NSF ATE Professional Development, *Genomic Approaches in Biosciences* Workshop, Shoreline Community College, Washington
	DNA Science Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Fun with DNA Workshop, DNALC *West*
	World of Enzymes Workshop, DNALC
July 1	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
July 5–8	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	Plant Genomics Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
July 5	HHMI Professional Development, *DNA Structure and Isolation* Workshop, *Harlem DNA Lab*
July 6	HHMI Professional Development, *Variability and Inheritance* Workshop, *Harlem DNA Lab*
July 7	HHMI Professional Development, *DNA Transformation and Protein Isolation* Workshop, *Harlem DNA Lab*
July 7–8	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, Washington University, St. Louis, Missouri
July 8	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
July 9–10	Botanical Society of America, "DNA Subway," and "DNA Barcoding," St. Louis, Missouri
July 11–15	*DNA Science* Workshop, DNALC
	DNA Science Workshop, DNALC *West*
	Human Genomics Workshop, Central Islip
	Human Genomics Workshop, DNALC
	World of Enzymes Workshop, DNALC
July 11	HHMI Professional Development, *DNA Transformation and Protein Isolation* Workshop, *Harlem DNA Lab*
July 12	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
July 13	HHMI Professional Development, *PCR and Human DNA Variation, Part 1* Workshop, *Harlem DNA Lab*
July 14	HHMI Professional Development, *PCR and Human DNA Variation, Part 2* Workshop, *Harlem DNA Lab*
July 15	HHMI Professional Development and *Urban Barcoding Project* Training Session, "DNA Barcoding," *Harlem DNA Lab*
July 18–22	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	Green Genes Workshop, DNALC *West*
	Silencing Genomes Workshop, DNALC
July 18–29	HHMI Professional Development, Leadership Symposium, *Harlem DNA Lab*
July 25–29	NSF Professional Development, *Silencing Genomes* Workshop, DNALC
	Forensic Detectives Workshop, DNALC
	Genetic Horizons Workshop, DNALC *West*

July 25–Aug. 12	*Introduction to Genetics and Biochemistry, DNA Science*, and *Genome Science* Workshops, Beijing No. 166 High School, DNALC
July 26	*Learn About Spinal Muscular Atrophy* interviews with Phil Sharp, Massachusetts Institute of Technology, Cambridge, and Richard Roberts, New England BioLabs Inc., Ipswich, Massachusetts
July 29	HHMI Professional Development and *Urban Barcoding Project* Training Session, "DNA Barcoding," *Harlem DNA Lab*
August 1–5	*Fun with DNA* Workshop, DNALC *West*
	Green Genes Workshop, DNALC
	World of Enzymes Workshop, DNALC
August 1	HHMI Professional Development, *DNA Structure and Isolation* Workshop, *Harlem DNA Lab*
August 2	HHMI Professional Development, *Variability and Inheritance* Workshop, *Harlem DNA Lab*
August 3	HHMI Professional Development, *DNA Transformation and Protein Isolation* Workshop, *Harlem DNA Lab*
August 4	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
August 5	HHMI Professional Development, *PCR and Human DNA Variation, Part 1* Workshop, *Harlem DNA Lab*
	Urban Barcode Project Training Session, New York Hall of Science, Queens, New York
August 8	HHMI Professional Development, *DNA Transformation and Protein Isolation* Workshop, *Harlem DNA Lab*
	American Society of Plant Biologists Annual Conference, "DNA Subway," Minneapolis, Minnesota
August 8–12	NSF ATE Professional Development, *Genomic Approaches in Biosciences* Workshop, Universidad del Turabo, Gurabo, Puerto Rico
	Genetic Horizons Workshop, DNALC
	World of Enzymes Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
August 9	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
August 10	HHMI Professional Development, *PCR and Human DNA Variation, Part 1* Workshop, *Harlem DNA Lab*
August 11	HHMI Professional Development, *PCR and Human DNA Variation, Part 2* Workshop, *Harlem DNA Lab*
August 12	HHMI Professional Development, *DNA Barcoding* Workshop, *Harlem DNA Lab*
	Urban Barcode Project Training Session, *Harlem DNA Lab*
	Learn About Spinal Muscular Atrophy interviews with Darryl De Vivo, Columbia Medical Center, and SMA patients and families, New York
August 15–19	NSF ATE Professional Development, *Genomic Approaches in Biosciences* Workshop, Madison Area Technical College, Wisconsin
	DNA Science Workshop, DNALC
	DNA Science Workshop, Stony Brook University, New York
	DNA Science Workshop, DNALC *West*
	Fun with DNA Workshop, DNALC
	Green Genes Workshop, DNALC
August 16	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
August 19	Site visit by Maryann Martone, Spinal Muscular Atrophy Foundation, New York
August 22–26	*DNA Science* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Genome Science Workshop, Stony Brook University, New York
	Human Genomics Workshop, DNALC
	Human Genomics Workshop, DNALC *West*
August 23	*Urban Barcode Project* Training Session, Genspace, Brooklyn, New York
August 26	Site visit by Pat Hayot, Michael Maloy, and Jill Hirsch, The Chapin School, New York
Aug. 29–Sept. 2	*DNA Science* Workshop, DNALC
	Green Genes Workshop, DNALC
	Green Genes Workshop, DNALC *West*
	World of Enzymes Workshop, DNALC
September 1	*Urban Barcode Project* Information Session, New York Botanical Garden, Bronx, New York
September 7	*Urban Barcode Project* Information Session, Richard Gilder Graduate School, American Museum of Natural History, New York
September 16	*Urban Barcode Project* Information Session, Trinity High School, New York
September 17	*Urban Barcode Project* Promotion at Maker Faire, New York Hall of Science, Queens, New York
	Graduate Student Training for STEM Mentoring Program, New York Academy of Science, New York
September 19	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
September 21	*Urban Barcode Project* Information Session, The Gateway Institute, New York
	Urban Barcode Project Information Session, Brooklyn Bridge Park, Brooklyn New York
September 23	*Urban Barcode Project* Information Session, American Museum of Natural History, New York
September 26	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
September 26–28	Site visit by Tony Chiovitti, Gene Technology Access Centre, Melbourne, Australia
September 28	*Urban Barcode Project* Information Session, Trinity High School, New York

October 1	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
October 3	Site visit by James Bono, Buck Koonce, and Camille Bibeau, Lawrence Livermore National Laboratory, Livermore, California
October 5	*Urban Barcode Project* Information Session, The River Project, New York University, New York
October 6	Pandora "Town Hall" Discussion and Reception, DNALC
	Urban Barcode Project Information Session, Columbia Secondary School for Mathematics, Science and Engineering, New York
October 7	Site visit by Kim Kerns and Katya Lanfant, Cablevision, New York
October 8	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
October 11–12	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, University of California, Riverside
October 12	*Saturday DNA!* "Superbugs Uncovered," DNALC
	Urban Barcode Project Information Session, Graduate Center, City University of New York, New York
October 12–15	NABT Professional Development Conference, "DNA Barcoding in Your Classroom," "Silencing Genomes," "RNAi/CSBC," "DNA Subway," "DNA Barcoding," and "Evaluation of Biology Teaching Resources," Anaheim, California
October 14	*Urban Barcode Project* Information Session, The Urban Assembly, New York
	HHMI Professional Development, *Variability and Inheritance* Workshop, *Harlem DNA Lab*
October 15	HHMI Professional Development, *Variability and Inheritance* Workshop, *Harlem DNA Lab*
October 21	HHMI Professional Development, *DNA Transformation and Protein Isolation* Workshop, *Harlem DNA Lab*
October 22	HHMI Professional Development, *DNA Transformation and Protein Isolation* Workshop, *Harlem DNA Lab*
October 24	Site visit by Ania Wieczorek, University of Hawaii, Manoa, Hawaii
October 25	Site visit by Mathias Schmitt, ARTE, Strasbourg Cedex, France
October 26–28	NSF ATE Principal Investigators Conference, "Developing Leaders in Biosciences," Washington, D.C.
October 27	*Urban Barcode Project* Information Session, Brooklyn Technical High School, Brooklyn, New York
October 28–29	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, Arnold Arboretum of Harvard University, Roslindale, Massachusetts
November 1	*Urban Barcode Project* Information Session, Department of Education, New York
November 3–4	Site visit by Shannon Oliver, *iPlant Collaborative*, Tucson, Arizona
November 5	*Urban Barcode Project* Training Session, Genspace, Brooklyn, New York
November 8	HHMI Professional Development, *DNA Structure and Isolation* Workshop, *Harlem DNA Lab*
	HHMI Professional Development, *DNA Structure and Isolation* Workshop, Genspace, Brooklyn, New York
November 10	Criminal Courts Bar Association of Nassau County, *DNA Analysis and Forensics* Workshop, Reception, and "DNA—Nothing to Fear," Eric Carita, Forensic DNA Consultant, DNALC
November 11	*Urban Barcode Project* Training Session, *Harlem DNA Lab*
November 11–12	NSF *iPlant Collaborative* Professional Development, *Genomics in Education* Workshop, University of Lone Star College, Kingwood, Texas
November 12	*Saturday DNA!* "Gram-tastic!" DNALC
November 14	*Urban Barcode Project* Information Session, Benjamin C. Cardozo High School, New York
November 15–16	NSF *iPlant Collaborative* Professional Development, *Tools and Services* Workshop, National Evolutionary Synthesis Center, Durham, North Carolina
November 16	Site visit by Siobhan McKenna, Agilent, Santa Clara, California
	Learn About Spinal Muscular Atrophy interview with Charlotte Sumner,The Johns Hopkins Hospital, Baltimore, Maryland
November 17–18	National Human Genome Research Institute (NHGRI) Genomic Literacy Workshop, "Limited Effects and Genome Education in the Digital Age," Rockville, Maryland
November 18	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
November 19	HHMI Professional Development, *DNA Analysis and Forensics* Workshop, *Harlem DNA Lab*
Nov. 28–Dec. 10	Singapore Primary Teachers Attachment, DNALC
November 30	NSF *iPlant Collaborative* Professional Development, *Tools and Services* Workshop, CSHL
Nov. 30–Dec. 2	International Barcode of Life Conference, "*Urban Barcode Project*: A Scalable Infrastructure for IBOL Outreach," Oaks Embassy, Adelaide, Australia
December 1	Site visit by Suzana Dobre, Mircea Miclea, and Roxana Vitan, Romanian-American Foundation, Bucharest, Romania
December 8	Site visit by Amanda Dubrowski, Bard College, Annandale on Hudson, New York
December 9	HHMI Professional Development, *PCR and Human DNA Variation, Part 1* Workshop, *Harlem DNA Lab*
December 10	*Saturday DNA!* "Mendelian Inheritance," DNALC
	HHMI Professional Development, *PCR and Human DNA Variation, Part 1* Workshop, *Harlem DNA Lab*
December 14	Site visit by Alex Wolfe, Second Nature Toys, New York

Sites of Major Faculty Workshops 1985–2011

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
	University of Arizona, Tucson	**2011**
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
	City College of San Francisco	**2011**
	Contra Costa County Office of Education, Pleasant Hill	2002, 2009
	Foothill College, Los Altos Hills	**1997**
	Harbor-UCLA Research & Education Institute, Torrance	2003
	Laney College, Oakland	1999
	Los Angeles Biomedical Research Institute (LA Biomed), Torrance	**2006**
	Lutheran University, Thousand Oaks	1999
	Oxnard Community College, Oxnard	2009
	Pasadena City College	**2010**
	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001, 2008
	San Francisco State University	**1991**
	San Jose State University	**2005**
	Santa Clara University, Santa Clara	2010
	University of California, Berkeley	**2010**
	University of California, Davis	1986
	University of California, Northridge	**1993**
	University of California, Riverside	2011
COLORADO	Aspen Science Center	2006
	Colorado College, Colorado Springs	1994, 2007
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998, 2009–2010
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
DISTRICT OF COLUMBIA	**Howard University, Washington**	**1992, 1996, 2009–2010**
FLORIDA	Armwood Senior High School, Tampa	1991
	Florida Agricultural & Mechanical University, Tallahassee	2007–2008
	Florida Agricultural & Mechanical University, Tallahassee	**2011**
	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
	Gwinnett Technical College, Lawrenceville	**2011**
	Morehouse College, Atlanta	**1991, 1996–1997**
	Spelman College, Atlanta	**2010**
HAWAII	Kamehameha Secondary School, Honolulu	1990
ILLINOIS	Argonne National Laboratory, Argonne	1986–1987
	iBIO Institute/Harold Washington College, Chicago	2010
	Illinois Institute of Technology, Chicago	2009
	University of Chicago	**1992, 1997, 2010**
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, Murray	1988
	University of Kentucky, Lexington	1992

	Western Kentucky University, Bowling Green	1992
LOUISIANA	Bossier Parish Community College, Bossier City	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
	Bowie State University, Bowie	**2011**
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools, Rockville	1990–1992
	National Center for Biotechnology Information, Bethesda	2002
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
MASSACHUSETTS	**Arnold Arboretum of Harvard University, Roslindale**	**2011**
	Beverly High School	1986
	Biogen Idec, Cambridge	2002, 2010
	Boston University	**1994, 1996**
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School, Randolph	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
	University of Minnesota, St. Paul	**2010**
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990–1991
	Rust College, Holly Springs	**2006–2008, 2010**
MISSOURI	**St. Louis Science Center**	**2008–2010**
	Stowers Institute for Medical Research, Kansas City	2002, 2008
	Washington University, St. Louis	**1989, 1997, 2011**
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	Bio-Link 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
	Canisius College, Buffalo	**2011**
	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 *West*, Lake Success	2005
	Dolan DNA Learning Center, Cold Spring Harbor	1988–1995, 2001–2004, 2006–2009
	Dolan DNA Learning Center, Cold Spring Harbor	**1990, 1992, 1995, 2000–2011**
	Dolan DNA Learning Center, Cold Spring Harbor	*1990–1992*
	Fostertown School, Newburgh	*1991*
	Harlem DNA Lab, East Harlem	2008–2009, 2011
	Huntington High School	1986
	Irvington High School	1986
	John Jay College of Criminal Justice, New York	2009

	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mount 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
	The Rockefeller University, New York	**2010**
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Saranac Lake	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, 2009–2011**
	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	Langston University, Langston	2008
	North Westerville High School	1990
OKLAHOMA	**Oklahoma City Community College**	**2000**
	Oklahoma City Community College	2006–2007, 2010
	Oklahoma Medical Research Foundation, Oklahoma City	2001
	Oklahoma School of Science and Math, Oklahoma City	1994
	Tulsa Community College	2009
OREGON	Kaiser Permanente–Center for Health Research, Portland	2003
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
	Kimmel Cancer Center, Philadelphia	2008
RHODE ISLAND	Botanical Society of America, Providence	2010
SOUTH CAROLINA	**Clemson University**	**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	**2009–2010**
	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	University of Lone Star College, Kingwood	**2011**
	Midland College	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, 2010**
	University of Texas, Brownsville	**2010**
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
	Shoreline Community College, Shoreline	2011
	University of Washington, Seattle	**1993, 1998, 2010**
WEST VIRGINIA	Bethany College, Bethany	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003
	Madison Area Technical College	**1999, 2009, 2011**
	Marquette University, Milwaukee	1986–1987
	University of Wisconsin, Madison	1988–1989
	University of Wisconsin, Madison	**2004**
WYOMING	University of Wyoming, Laramie	1991
PUERTO RICO	**Universidad del Turabo, Gurabo, Puerto Rico**	**2011**
	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

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	International Institute of Genetics and Biophysics, Naples	1996
	Porto Conte Research and Training Laboratories, Alghero	1993
MEXICO	ASPB Plant Biology, Merida	2008
PANAMA	**University of Panama, Panama City**	**1994**
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001–2005
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University	2000
THE NETHERLANDS	**International Chromosome Conference, Amsterdam**	**2007**

COLD SPRING HARBOR
LABORATORY PRESS

2011 PRESS PUBLICATIONS

Serials

Genes & Development, Vol. 25 (www.genesdev.org)

Genome Research, Vol. 21 (www.genome.org)

Learning & Memory, Vol. 18 (www.learnmem.org)

RNA, Vol. 17 (www.rnajournal.org)

Cold Spring Harbor Symposia on Quantitative Biology, Vol. 75: *Nuclear Organization and Function,* edited by Terri Grodzicker, David Spector, and Bruce Stillman

Cold Spring Harbor Protocols (www.cshprotocols.org)

Cold Spring Harbor Perspectives in Biology (www.cshperspectives.org)

Cold Spring Harbor Perspectives in Medicine (www.perspectivesinmedicine.org)

Laboratory Manuals

Imaging in Neuroscience: A Laboratory Manual, edited by Fritjof Helmchen and Arthur Konnerth; Series Editor Rafael Yuste

Textbooks

An Introduction to Animal Behavior: An Integrative Approach, by Michael J. Ryan and Walter Wilcynski

Yeast Intermediary Metabolism, by Dan G. Fraenkel

Monographs (Topic Collections from *Perspectives in Biology* and *Perspectives in Medicine*)

The Biology of Lipids: Trafficking, Regulation, and Function, edited by Kai Simons

The Mammary Gland as an Experimental Model, edited by Mina J. Bissell, Kornelia Polyak, and Jeffrey M. Rosen

Germ Cells, edited by Paolo Sassone-Corsi, Margaret T. Fuller, and Robert Braun

The Golgi, edited by Graham Warren and James Rothman

Calcium Signaling, edited by Martin D. Bootman, Michael J. Berridge, James W. Putney, and H. Llewelyn Roderick

Protein Homeostasis, edited by Richard I. Morimoto, Dennis J. Selkoe, and Jeffery W. Kelly

Extracellular Matrix Biology, edited by Richard O. Hynes and Kenneth M. Yamada

Angiogenesis: Biology and Pathology, edited by Michael Klagsbrun and Patricia A. D'Amore

The Biology of Alzheimer Disease, edited by Dennis J. Selkoe, Eckhard Mandelkow, and David M. Holtzman

HIV: From Biology to Prevention and Treatment, edited by Frederic D. Bushman, Gary J. Nabel, and Ronald Swanstrom

History

Mutation: The History of an Idea from Darwin to Genomics, by Elof Axel Carlson

RNA: Life's Indispensable Molecule, by James Darnell

Science and Society

Incurable: A Life After Diagnosis, by Charles Harris

Other

CSHL Annual Report 2010, Yearbook Edition

CSHL Annual Report 2010, Executive Summary

Banbury Center Annual Report 2010

Watson School of Biological Sciences Annual Report 2010

Online Book

Guide to the Human Genome, by Stewart Scherer (www.cshlp.org)

E-books (Kindle editions)

The Honest Look, by Jennifer L. Rohn

Sydney Brenner: A Biography, by Errol F. Friedberg

Imaging: A Laboratory Manual, edited by Rafael Yuste

At the Helm: Leading Your Laboratory, Second Edition, by Kathy Barker

At the Bench: A Laboratory Navigator, Updated Edition, by Kathy Barker

Websites

Cold Spring Harbor Monograph Archive (www.cshmonographs.org)

Cold Spring Harbor Symposium on Quantitative Biology Archive (symposium.cshlp.org)

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

The publications of Cold Spring Harbor Laboratory Press are used and valued throughout the world, a reminder that the Laboratory has a special place in the landscape of international science. Nowhere else combines research excellence with professional education of the nature and quality that Cold Spring Harbor offers. The research and review journals, handbooks, manuals, monographs, histories, biographies, and textbooks of the Press are a vital part of how the Laboratory connects with the worldwide scientific community. Our goal is to identify important research, technologies, and scientists; to amplify their effectiveness by selecting, aggregating, and curating information research communities can use; and to deliver that information when, where, and how the communities want it. By successfully achieving these goals, the Press makes a substantial financial contribution to the Laboratory. In 2011, that contribution exceeded $1.4 million, the largest the Press has ever made. The major drivers of this success were the continued subscription growth among our journals, both mature and start-up titles, and the cost-cutting measures that were implemented at the end of 2010.

During the year, research articles published by the Press' seven scientific journals were downloaded more than 8.8 million times, more than 2100 electronic journal subscriptions were fulfilled at academic institutions in 160 countries (including all leading American universities), and 39,600 copies of print books were shipped. This reach is complemented by interest from foreign publishers in translating certain books into languages such as Chinese, Japanese, Arabic, and, most recently, Vietnamese.

As measured by impact factor, the best-known metric of worth, all of our journals advanced in 2011. *Genes & Development* and *Genome Research* continue to occupy the first tier of genetics, biotechnology, and developmental biology journals, and of the 8000 most important science journals published in the world, they are within the top 1.2%. All of the journals exceeded financial expectation and *Cold Spring Harbor Protocols*, in its fifth year, had a particularly remarkable increase in subscriptions. A new journal, *Cold Spring Harbor Perspectives in Medicine*, was launched on schedule in September, with papers on AIDS, Alzheimer's disease, and Parkinson's syndrome. This new online-only review journal will have a wide scope, ranging from the molecular and cellular basis of disease to translational medicine and emerging therapeutic strategies.

Seventeen new books were published during the year, bringing the number of available titles to more than 150. James Darnell's book, *RNA: Life's Indispensable Molecule*, published in July to critical acclaim, was named one of the 25 Outstanding Academic Titles for 2011 by *Choice* magazine. Elof Carlson's *Mutation: From Darwinian Fluctuations to Comparative Genomics* was also warmly received by reviewers. The best-selling titles of the year were the classic handbooks *At the Bench* and *At the Helm,* the iconic manual *Molecular Cloning*, and the magisterial history of molecular biology *The Eighth Day of Creation* by Horace Freeland Judson, whose passing at the age of 80 during the year we noted with great sadness. Our mission to deliver scientific information when, where, and how it is needed is being profoundly altered by changes in distribution channels. Laboratory protocols and topic-specific review articles are two types of content for which the Press has been renowned. Five years ago, that content was delivered only in printed books. Now, it comes also in the much more usable form of online-only subscription-based review journals made possible by the reputation of those print books and the strategies used in creating them. So the same content types now have an additional means of delivery and a different, more robust business model—but the same exceptional quality. Print book sales have declined but our journal subscriptions, particularly for review journals, are rising. We are adapting our strategy, our technology, and our staffing structure to take

account of this digital evolution. E-books and device apps are being explored. All Press journals online are now optimized for reading on mobile phones and tablet devices. Although so far, as readers, scientists are not abandoning the familiar and substituting with these new media formats, these are still early days. More than 6000 people are now following our journals on Twitter, a dramatic increase in a year. It is an exhilarating time to be engaged in scientific communication and Cold Spring Harbor Laboratory Press is committed to maintaining its reputation and effectiveness in the rapidly changing world of digital information.

Staff

During the year, we said farewell to David Crotty, Antonella Caiazza, Victor Manchiso, and Ann Smith. A particularly notable event in May was the retirement of Bill Keen from his position as Finance Director, a position he had occupied with great effectiveness for 10 years. For the previous 30 years, however, Bill had been the Laboratory's Comptroller, the most senior position in the institution's financial management. He served in this role during a period of extraordinary growth in the size and complexity of the Laboratory, and his calm and good humor in managing CSHL's internal affairs as well as its engagement with the wider financial world were much appreciated by the Laboratory's leadership and Board of Trustees at the time. Replacing his vital contribution to the management of the Press was a formidable challenge. However, we were fortunate to be able to recruit Stephen Nussbaum to join us in April, bringing with him an impressive degree of experience gained in senior positions in New York publishing, most recently at Oxford University Press. His credentials also include a CUNY teaching position in accountancy. Steve has quickly become indispensable to our operations. We also welcomed Michael Henigman, Diane Schubach, Richard Abreu, and Matthew D'Amico. In March, Richard Sever was promoted to assistant director of the Press, a newly created position in which he will assist in the strategic management of the organization while continuing his editorial activities for the *Perspectives* and *Protocols* journals and the books program. Since he joined us 3 years ago, Richard's energy, productivity, and ideas have helped us make significant improvements in output and internal efficiency, and it is a pleasure to have his talents in this expanded role.

A complete list of staff members of the Press at the end of December 2011 is printed elsewhere in this volume. Publishing is a team-based activity, reliant on the effective meshing of groups of people with a variety of skills. The Press is fortunate to have able and dedicated individuals in all its departments. But I am particularly grateful to my hard-working and unfailingly cheerful assistant Mala Mazzullo and to the talented individuals who have leadership roles in the activities of the Press: Jan Argentine, Alex Gann, Terri Grodzicker, Wayne Manos, Stephen Nussbaum, 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, 2011
(with comparative financial information as of December 31, 2010)

	2011	2010
Assets:		
Cash and cash equivalents	$ 58,055,329	60,766,362
Grants receivable	5,733,104	7,725,659
Contributions receivable, net	163,144,645	104,272,591
Publications inventory	3,292,898	3,555,193
Investments	269,786,326	276,424,730
Restricted use assets	2,882,590	2,580,471
Other assets	15,027,787	9,922,097
Land, buildings and equipment, net	241,828,796	238,777,879
Total assets	$ 759,751,475	704,024,982
Liabilities and net assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 11,135,584	9,195,850
Deferred revenue	5,467,566	8,210,668
Interest rate swap	37,726,697	16,439,159
Bonds payable	97,200,000	97,200,000
Total liabilities	151,529,847	131,045,677
Net assets:		
Unrestricted	205,967,407	227,854,292
Temporarily restricted	298,351,339	242,589,902
Permanently restricted	103,902,882	102,535,111
Total net assets	608,221,628	572,979,305
Total liabilities and net assets	$ 759,751,475	704,024,982

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2011
(with summarized financial information for the year ended December 31, 2010)

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2011 Total*	*2010 Total*
Revenue and other support:					
Public support–contributions and nonfederal grant awards	$ 19,694,851	92,621,395	1,367,771	113,684,017	45,420,328
Federal grant awards	34,490,267	–	–	34,490,267	35,405,944
Indirect cost allowances	25,623,148	–	–	25,623,148	25,973,354
Investment return utilized	13,114,916	9,470,558	–	22,585,474	18,309,184
Program fees	6,963,110	–	–	6,963,110	6,971,358
Publications sales	9,848,446	–	–	9,848,446	9,251,441
Dining services	4,309,694	–	–	4,309,694	4,319,847
Rooms and apartments	3,345,273	–	–	3,345,273	3,556,328
Miscellaneous	3,745,958	–	–	3,745,958	2,126,513
Net assets released from restrictions	36,474,563	(36,474,563)	–	–	–
Total revenue and other support	157,610,226	65,617,390	1,367,771	224,595,387	151,334,297
Expenses:					
Research	91,848,784	–	–	91,848,784	86,810,119
Educational programs	16,117,941	–	–	16,117,941	17,121,875
Publications	9,323,927	–	–	9,323,927	10,877,055
Banbury Center conferences	1,409,384	–	–	1,409,384	1,370,778
DNA Learning Center programs	1,857,078	–	–	1,857,078	1,406,339
Watson School of Biological Sciences programs	3,584,716	–	–	3,584,716	3,535,758
General and administrative	115,596,163	–	–	15,596,163	14,746,870
Dining services	5,717,278	–	–	5,717,278	5,137,648
Total expenses	145,455,271	–	–	145,455,271	141,006,442
Excess of revenue and other support over expenses	12,154,955	65,617,390	1,367,771	79,140,116	10,327,855
Other changes in net assets:					
Investment (loss) return excluding amount utilized	(12,754,302)	(9,855,953)	–	(22,610,255)	8,599,598
Change in fair value of interest rate swap	(21,287,538)	–	–	(21,287,538)	(4,990,800)
(Decrease) increase in net assets	(21,886,885)	55,761,437	1,367,771	35,242,323	13,936,653
Net assets at beginning of year	227,854,292	242,589,902	102,535,111	572,979,305	559,042,652
Net assets at end of year	$ 205,967,407	298,351,339	103,902,882	608,221,628	572,979,305

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2011
(with comparative financial information for the year ended December 31, 2010)

	2011	2010
Cash flows from operating activities:		
Increase in net assets	$ 35,242,324	13,936,653
Adjustments to reconcile increase in net assets to net cash (used in) provided by operating activities:		
Change in fair value of interest rate swap	21,287,538	4,990,800
Depreciation and amortization	14,697,263	15,443,523
Net depreciation (appreciation) in fair value of investments	4,173,189	(23,724,939)
Contributions restricted for long-term investment	(13,475,213)	(1,712,498)
Changes in assets and liabilities:		
Grants receivable	1,992,555	(1,525,261)
Contributions receivable, net of financing activities	(53,517,765)	11,309,672
Publications inventory	262,295	1,015,085
Other assets	(5,008,296)	929,237
Restricted use assets	(302,119)	(469,381)
Accounts payable and accrued expenses, net of financing activities	1,835,064	(2,521,650)
Deferred revenue	(2,743,102)	2,570,639
Net cash provided by operating activities	(4,443,733)	20,241,880
Cash flows from investing activities:		
Capital expenditures	(17,748,180)	(16,429,996)
Proceeds from sales and maturities of investments	33,325,960	36,404,485
Purchases of investments	(30,860,745)	(47,508,712)
Net change in investment in employee residences	(97,394)	(453,121)
Net cash used in investing activities	(15,380,359)	(27,987,344)
Cash flows from financing activities:		
Contributions restricted for long-term investment	1,600,563	1,109,499
Contributions restricted for investment in capital	11,874,650	602,999
(Increase) decrease in contributions receivable	(5,354,289)	4,157,922
Increase (decrease) in accounts payable relating to capital expenditures	104,669	(3,309,152)
Net cash provided by financing activities	8,225,593	2,561,268
Net decrease in cash and cash equivalents	(2,711,033)	(5,184,196)
Cash and cash equivalents at beginning of year	60,766,362	65,950,558
Cash and cash equivalents at end of year	$ 58,055,329	60,766,362
Supplemental disclosure:		
Interest paid	$ 3,614,370	3,652,690
Noncash investing and financing activity:		
Contributed property	$ 1,095,000	57,475

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the 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 2011.

GRANTS January 1–December 31, 2011

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]	
FEDERAL GRANTS					
NATIONAL INSTITUTES OF HEALTH					
Program Project and Center Support	Drs. Hannon/Krainer/Lowe/Spector/Stillman	01/01/07	12/31/12	$ 4,592,515	
	Dr. Stillman–Cancer Center Core	08/17/11	07/31/16	4,306,037	*
Cooperative Research Agreement Support[2]	Drs. Gingeras/Hannon	09/27/07	06/30/12	2,037,906	
	Drs. Lowe/Hannon/Hicks/Powers	09/01/09	08/31/14	841,281	
Research Support	Dr. Churchland	09/01/10	08/31/13	249,801	
	Dr. Dubnau	09/15/09	08/31/14	430,650	
	Drs. Enikolopov/Koulakov	09/15/11	08/31/16	437,918	*
	Dr. Furukawa	03/01/10	02/28/15	430,650	
	Dr. Hannon	09/01/09	08/31/13	349,602	
	Dr. Huang	07/01/11	03/31/16	556,080	*
	Dr. Joshua-Tor	07/01/07	06/30/12	304,484	
	Drs. Kepecs/Huang	07/01/11	05/31/16	388,063	*
	Dr. Koulakov	02/01/08	01/31/13	413,424	
	Drs. Koulakov/Enikolopov	07/15/10	02/28/14	334,485	
	Dr. Li	07/01/10	03/31/15	518,880	
	Dr. Martienssen	09/15/11	08/30/15	354,798	*
	Dr. McCombie	07/23/10	02/28/15	1,039,526	
	Dr. Mills	12/26/07	11/30/12	351,641	
	Dr. Mitra	08/01/10	05/31/13	376,587	
	Drs. Mitra/Osten	09/30/09	03/31/14	914,483	
	Dr. Muthuswamy	02/01/09	12/31/13	377,711	
	Dr. Powers	12/02/06	11/30/12	19,310	
	Dr. D. Spector	04/01/11	03/31/15	687,053	*
	Dr. Stenlund	02/01/08	01/31/13	426,344	
	Dr. Stillman	06/01/08	05/31/12	638,144	
	Dr. Tonks	07/01/10	04/30/15	687,770	
	Dr. Tonks	01/11/10	12/31/13	404,432	
	Dr. Trotman	04/01/10	01/31/14	240,475	
	Dr. Turner	07/15/10	06/30/15	421,080	
	Dr. Van Aelst	07/01/08	03/31/13	387,585	
	Dr. Van Aelst	01/01/09	12/31/13	350,219	
	Dr. Zador	09/18/09	07/31/13	354,339	
	Dr. Zador	09/27/10	05/31/15	430,650	
	Dr. Zhong	06/01/10	05/31/13	341,040	

[1]Includes direct and indirect costs
[2]Cooperative research agreement funding amounts include only CSHL portion of award
*New or competing renewal grants awarded in 2011

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]
Research Subcontracts				
NIH/Allen Institute for Brain Science Consortium Agreement	Dr. Mitra	09/15/09	08/31/13	$ 244,200
NIH/Carnegie Mellon University Consortium Agreement	Dr. Osten	09/15/10	07/31/13	113,100
NIH/Cornell University Consortium Agreement	Dr. Mitra	04/03/08	01/31/12	20,235
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	07/31/15	292,219
NIH/University of California, Berkeley National Laboratory Consortium Agreement	Dr. Gingeras	05/01/07	03/31/12	205,801
NIH/University of California, San Francisco Consortium Agreement	Dr. Egeblad	09/01/09	08/31/14	182,652
NIH/University of Southern California Consortium Agreement	Dr. Hannon	07/01/10	06/30/14	117,450
NIH/University of Texas Consortium Agreement	Dr. Furukawa	09/30/11	05/31/15	51,819 *
NIH/University of Texas Consortium Agreement	Dr. Krainer	08/01/10	07/31/12	78,839
Fellowship Support	Dr. Borges	08/16/09	08/15/12	54,734
	Dr. Ipsaro	09/01/11	08/31/13	48,398 *
	Dr. Jansen	01/01/11	12/31/13	48,398 *
Institutional Training Program Support	Dr. Joshua-Tor, Watson School of Biological Sciences, Predoctoral	07/01/07	06/30/12	251,640
	Dr. Mills, Postdoctoral	09/01/11	08/31/16	175,032 *
Course Support	Advanced Immunocytochemistry: In Situ Hybridization and Live Cell Imaging	09/01/10	08/31/15	100,166
	Advanced Techniques in Molecular Neuroscience	07/01/01	06/30/12	81,866
	Cell and Developmental Biology of *Xenopus*	04/01/09	03/31/14	56,335
	Cellular Biology of Addiction	03/01/11	02/29/16	79,300 *
	Computational and Comparative Genomics	06/06/91	07/31/13	51,792
	Imaging Structure and Function in the Nervous System	07/01/01	06/30/12	77,520
	Integrated Data Analysis for High Throughput Biology	09/01/07	04/30/12	81,564
	Neurobiology of *Drosophila*	07/01/01	06/30/12	61,508
	Programming for Biology	09/01/09	08/31/14	63,654
	X-Ray Methods in Structural Biology	07/01/07	06/30/12	77,830
	Yeast Genetics and Genomics	09/18/10	06/30/13	53,462
Meeting Support	The Biology of Cancer: Microenvironment, Metastasis and Therapeutics	04/01/11	03/31/12	3,000 *
	The Biology of Genomes	04/01/08	03/31/13	39,339
	Cell Death	09/14/07	08/31/12	4,000
	Eukaryotic DNA Replication and Genome Maintenance	09/01/11	08/31/12	6,500 *
	Eukaryotic mRNA Processing	06/03/11	05/31/12	4,000 *
	Genome Informatics	08/08/11	07/31/12	30,990 *
	Harnessing Immunity to Prevent and Treat Disease	11/04/11	10/31/12	10,000 *

[1]Includes direct and indirect costs
*New or competing renewal grants awarded in 2011

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]
	Metabolism and Disease	05/30/11	04/30/12	$ 28,000 *
	Microbial Pathogenesis and Host Response	08/15/11	07/31/12	15,000 *
	Network Biology	04/21/09	02/28/14	5,000
	Neurobiology of *Drosophila*	09/01/11	08/31/12	15,000 *
	PTEN Pathways and Targets	09/30/11	08/31/12	7,500 *
	Retroviruses	04/15/11	03/31/12	15,000 *
	Synapses: From Molecules to Circuits and Behavior	04/01/11	01/31/16	20,000 *
	The Ubiquitin Family	04/11/07	03/31/12	4,500
	Yeast Cell Biology	06/01/11	05/31/16	5,000 *
NATIONAL SCIENCE FOUNDATION				
Multiple Project Award Support	Dr. Jackson	10/01/10	09/30/15	1,071,458
	Drs. McCombie/Martienssen/Ware	08/01/10	07/31/12	999,985
Research Support	Dr. Churchland	08/01/11	07/31/14	43,155 *
	Dr. Jackson	09/01/10	08/31/13	150,000
	Dr. Lippman	09/15/09	08/31/12	172,910
	Dr. Timmermans	08/15/10	07/31/13	150,000
Research Subcontracts				
NSF/Cornell University Consortium Agreement	Dr. Timmermans	09/01/08	08/31/12	413,998
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	03/01/11	02/29/16	491,432 *
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	08/01/10	07/31/14	396,042
NSF/University of Arizona Consortium Agreement	Dr. Ware	08/15/10	07/31/14	605,604
NSF/University of Arizona Consortium Agreement	Drs. Ware/Martienssen/Stein	02/01/08	01/31/13	1,774,355
Facility Upgrade and Renovation	Drs. Martienssen Jackson/Lippman/ Timmermans/Ware	10/01/10	09/30/12	497,423 [3]
Fellowship Support	Dr. Liberatore	06/01/10	05/31/13	40,500
Undergraduate Training Support	Dr. Lippman	03/01/09	02/29/12	72,197
Course Support	Advanced Bacterial Genetics	07/01/09	06/30/14	93,258
	Computational Cell Biology	08/15/11	07/31/12	60,000 *
	Frontiers and Techniques in Plant Science	07/01/11	06/30/12	57,000 *
Meeting Support	Computational Cell Biology	03/15/11	02/29/12	20,950 *
	Eukaryotic DNA Replication and Genome Maintenance	08/01/11	07/31/12	7,000 *
	Eukaryotic mRNA Processing	08/01/11	07/31/12	7,000 *
	The Future of Plant Genome Sequencing and Analysis	05/01/11	04/30/12	48,840 *
	Mechanisms of Eukaryotic Transcription	08/01/11	07/31/12	7,000 *
	Neurobiology of *Drosophila*	08/15/11	07/31/12	21,220 *

[1]Includes direct and indirect costs
[3]Award issued under the American Recovery and Reinvestment Act of 2009
*New or competing renewal grants awarded in 2011

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]	
Meeting Support (continued)	Plant Biology Conference	09/15/11	08/31/12	$ 30,000	*
	Yeast Cell Biology	08/01/11	07/31/12	10,528	*
Meeting Subcontracts					
NSF/University of Illinois	Honey Bee Genomics and Biology	04/01/11	03/31/12	25,000	*
UNITED STATES DEPARTMENT OF AGRICULTURE					
Research Support	Dr. Jackson	01/15/11	01/14/15	119,117	*
	Dr. McCombie	09/11/09	09/10/14	268,198	
UNITED STATES DEPARTMENT OF ENERGY					
Research Support	Dr. Martienssen	09/03/10	09/02/12	797,433	
Research Subcontracts					
DOE/Brookhaven National Laboratory	Drs. Ware/Schatz	11/29/11	09/30/16	1,050,000	*
UNITED STATES DEPARTMENT OF THE ARMY					
Research Support	Dr. Hannon	09/01/08	08/31/13	687,346	
	Dr. Trotman	08/15/09	08/14/12	88,546	
	Drs. Wigler/Hicks/Krasnitz	09/01/11	08/31/12	2,140,000	*
	Dr. Zhong	06/15/10	06/14/13	293,951	
Fellowship Support	Dr. Nakasone	01/01/11	12/31/13	43,200	*
	Dr. B. Zhang	03/15/10	03/14/12	62,428	
MISCELLANEOUS SOURCES OF FUNDING					
Equipment					
The Mary K. Chapman Foundation	Dr. McCombie	12/31/10	12/31/11	250,000	*
	Dr. Stillman	12/22/11	12/21/12	450,000	*
Alan Seligson	Dr. Wigler	06/01/11	05/31/12	100,000	*
Theodore R. and Vada S. Stanley	Drs. Watson/McCombie	06/01/11	05/31/14	5,000,000	*
Program Project Support					
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lippman/Martienssen/ Timmermans/Ware	07/01/07	06/30/12	1,600,000	
Pfizer Inc.	Dr. Stillman	12/31/11	12/31/13	1,600,000	*
Theodore R. and Vada S. Stanley	Drs. Watson/McCombie	07/01/07	06/30/12	5,000,000	
The Simons Foundation/Center for Quantitative Biology	Dr. Wigler	09/01/08	08/31/15	1,500,000	
Research Support					
Paul G. Allen Family Foundation	Dr. Zador	10/01/10	09/30/13	564,900	
Alzheimer's Association	Dr. Furukawa	03/01/10	02/28/12	30,031	
American Cancer Society	Dr. Wigler	01/01/11	12/31/11	10,000	*
	Dr. Wigler	01/01/11	08/31/12	70,000	*

[1]Includes direct and indirect costs
*New or competing renewal grants awarded in 2011

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]	
The A&P Foundation	Dr. Egeblad	06/01/11	05/31/12	$ 20,000	*
Babylon Breast Cancer Coalition Inc.	Dr. Egeblad	12/30/11	12/29/12	7,100	*
Breast Cancer Alliance	Dr. Egeblad	01/01/10	12/31/11	62,500	
The Breast Cancer Research Foundation	Dr. Wigler	10/01/11	09/30/12	225,000	*
Burroughs Wellcome Fund	Dr. Vakoc	09/01/11	08/31/16	95,000	*
Carnegie Mellon University/Anonymous	Dr. Osten	01/01/10	11/30/11	75,000	
The Mary K. Chapman Foundation	Dr. Osten	12/31/11	12/30/13	300,000	*
Mr. and Mrs. Edward Chernoff in memory of James J. Kehoe	Dr. Furukawa	08/15/10	08/14/11	500	
Clear Channel Worldwide (Walk 97.5)	Dr. Egeblad	06/01/11	05/31/12	4,500	*
The Dana Foundation	Dr. Li	01/01/09	12/31/11	200,000	
	Dr. Li	10/01/09	09/30/12	69,156	
Dart Neuroscience LLC	Dr. Dubnau	10/01/11	09/30/12	250,000	*
The Kathryn W. Davis Foundation	Dr. Hannon	01/15/07	01/14/12	986,640	
The Ellison Medical Foundation	Dr. Enikolopov	09/09/09	09/08/13	252,000	
Entertainment Industry Foundation	Dr. Hannon	11/01/11	10/31/12	250,000	*
The Eppley Foundation for Research, Inc.	Dr. Shea	07/01/11	06/30/12	50,000	*
Edward P. Evans Foundation	Dr. Vakoc	11/01/11	10/31/12	500,000	*
Charitable Lead Annuity Trust under the Will of Louis Feil	Dr. Osten	12/29/10	11/30/12	225,000	
Find a Cure Today Long Island Foundation	Dr. Egeblad	03/01/11	02/29/12	10,000	*
Douglas and Christine Fox	Dr. Zhong	12/15/11	12/14/12	1,000	*
The Joni Gladowsky Breast Cancer Foundation	Dr. Tonks	10/01/11	09/30/12	35,000	*
Glen Cove C.A.R.E.S., Inc.	Dr. Egeblad	02/01/11	01/31/12	10,000	*
	Dr. Sordella	02/01/11	01/31/12	10,000	*
Good Samaritan, Inc.	Dr. Li	06/01/11	05/31/12	75,000	*
Richard and Mindy Gordon	Dr. Egeblad	01/01/11	12/31/11	1,000	*
The Irving A. Hansen Memorial Foundation	Dr. Tonks	07/01/11	06/30/12	20,000	*
Jo-Ellen and Ira Hazan	Dr. Osten	12/01/11	11/30/12	200,000	*
Hearts for Cancer	Dr. Egeblad	04/01/11	03/31/12	8,473	*
Howard Hughes Medical Institute–Gordon and Betty Moore Foundation	Dr. Martienssen	12/01/11	11/30/16	333,333	*
The International Human Frontier Science Program	Dr. Lippman	07/01/09	06/30/12	100,000	
Isis Pharmaceuticals, Inc.	Dr. Krainer	11/01/11	10/26/13	150,000	*
	Dr. Krainer	06/15/11	06/14/12	50,000	*
Islip Breast Cancer Coalition Inc.	Dr. Tonks	11/15/11	11/14/12	10,000	*
Sidney Kimmel Foundation for Cancer Research	Dr. Zheng	07/01/11	06/30/13	100,000	*
F.M. Kirby Foundation, Inc.	Dr. Vakoc	09/15/11	09/14/12	100,000	*
The Susan G. Komen Breast Cancer Foundation, Inc.	Dr. Egeblad	04/13/10	04/12/13	150,000	
Lake Grove Triangle Soccer, Inc.	Dr. Vakoc	06/01/11	05/31/12	3,000	*
The George Link, Jr. Foundation, Inc.	Dr. Sordella	12/01/11	11/30/12	50,000	*
The Long Island 2-Day Walk to Fight Breast Cancer	Dr. Egeblad	08/15/11	08/14/12	28,000	*
The Lustgarten Foundation	Drs. Hannon/Lowe	01/01/10	12/31/12	1,042,732	
	Drs. Powers/Lowe/Van Aelst	02/01/10	01/31/12	467,921	
The Manhasset Women's Coalition Against Breast Cancer, Inc.	Dr. Egeblad	01/01/11	12/31/11	100,000	*
	Dr. Egeblad	07/19/11	07/18/12	1,000	*
Carol Marcincuk Fund	Dr. Tonks	01/01/11	12/31/11	7,380	*
In honor of Carissa Maringo	Dr. Egeblad	10/01/11	09/30/12	5,000	*

[1]Includes direct and indirect costs
*New or competing renewal grants awarded in 2011

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]	
Research Support (continued)					
The G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Mitra	09/01/10	08/31/13	$ 03,919	
Breast Cancer Awareness Day in memory of Elizabeth McFarland	Dr. Wigler	01/01/11	12/31/11	72,305	*
The John Merck Fund	Dr. Kepecs	06/01/10	05/31/14	75,000	
	Dr. Churchland	06/01/11	05/31/15	75,000	*
The Melanoma Research Alliance	Drs. Hannon/Hammell/Vakoc	08/01/11	07/31/14	200,000	*
Mirus Bio LLC	Dr. Furukawa	10/18/11	10/17/12	5,000	*
The Don Monti Memorial Research Foundation	Dr. Lowe	03/01/11	02/29/12	400,000	*
Louis Morin Charitable Trust	Dr. Joshua-Tor	12/01/11	11/30/12	60,000	*
MSC Biotarget LLC	Dr. Tonks	11/01/09	10/31/11	60,000	
NARSAD	Dr. Li	07/15/11	07/14/13	30,000	*
	Dr. Huang	10/15/11	10/14/12	100,000	*
New York State Department of Health–New York Stem Cell Science (NYSTEM)	Drs. Enikolopov/Koulakov	01/01/09	12/31/11	360,000	
Panera Bread/Doherty Breads, LLC	Dr. Egeblad	12/01/11	11/30/12	7,600	*
The Perkin Fund	Dr. Egeblad	01/01/11	12/31/11	75,000	*
The Pew Charitable Trusts	Dr. Hannon	01/01/11	12/31/15	348,074	*
V. Kann Rasmussen Foundation	Dr. Trotman	09/01/07	08/31/12	50,000	
Christina Renna Foundation Inc.	Dr. Van Aelst	01/01/11	12/31/11	15,000	*
Damon Runyon Cancer Research Foundation	Dr. Sordella	01/01/10	12/31/12	150,000	
Diane Emdin Sachs Memorial Fund	Dr. Sordella	09/01/11	08/31/12	8,949	*
Eleanor Schwartz Charitable Foundation	Dr. Churchland	07/15/11	07/14/14	200,000	*
Judi Shesh Memorial Foundation	Dr. Egeblad	09/01/11	08/31/12	5,000	*
	Dr. Tonks	09/01/11	08/31/12	5,000	*
The Simons Foundation	Dr. Henn	09/01/10	08/31/12	305,791	
	Dr. Henn	09/01/10	08/31/12	602,811	
	Dr. Mills	07/01/10	06/30/13	350,000	
	Drs. Mitra/Huang/Osten	12/01/11	11/30/14	300,000	*
	Dr. Osten	10/01/11	09/30/13	175,000	*
Spinal Muscular Atrophy Foundation	Dr. Krainer	09/01/11	08/31/12	100,000	*
Mary F. Smith Family Foundation	Dr. Egeblad	12/01/11	11/30/12	5,000	*
Starr Cancer Consortium	Dr. Hannon	08/01/11	07/31/13	216,000	*
	Dr. Hannon	08/01/11	07/31/13	270,000	*
	Drs. Hicks/Wigler	08/01/11	07/31/13	270,000	*
	Drs. Sordella/Pappin	08/01/11	07/31/13	249,301	*
	Drs. Sordella/Van Aelst	08/01/10	07/31/12	669,422	
	Dr. Sordella	08/01/11	07/31/13	216,000	*
	Dr. Vakoc	08/01/10	07/31/12	83,750	
	Dr. Vakoc	08/01/11	07/31/13	180,000	*
Swim Across America	Dr. Sordella	12/15/11	12/14/14	75,000	*
U.S.–Israel Binational Agricultural Research and Development Fund	Dr. Krainer	10/01/10	09/30/14	10,000	
	Dr. Lippman	06/01/10	05/31/13	42,000	
	Dr. Zador	10/01/10	09/30/14	13,000	
University Health Network	Dr. Muthuswamy	01/01/11	12/31/11	50,000	*
University of California, Los Angeles/ Entertainment Industry Foundation	Dr. Hannon	10/01/09	09/30/12	414,284	
The V Foundation	Dr. Zheng	11/01/11	10/31/13	100,000	*
West Islip Breast Cancer Coalition for Long Island Inc.	Dr. Egeblad	11/01/11	10/31/12	10,000	*

[1]Includes direct and indirect costs
*New or competing renewal grants awarded in 2011

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]
Whitehall Foundation, Inc.	Dr. Shea	09/01/11	08/31/14	$ 75,000 *
Wild for Zumba	Dr. Egeblad	12/01/11	11/30/12	1,013 *
Ms. Evelyn W. Willard	Dr. Churchland	12/21/10	12/20/11	50,000
The Bradley Zankel Foundation, Inc.	Dr. Zheng	07/16/11	07/15/12	15,000 *
Fellowship Support				
American Cancer Society	Dr. Feigin	01/01/11	12/31/12	50,000 *
	Dr. Preall	10/01/11	09/30/14	48,000 *
Australian Government National Health and Medical Research Council	Dr. Bolden	04/01/10	03/31/12	39,155
	Dr. Dow	02/01/10	01/31/11	13,640
Carnegie Institution for Science	Dr. Timmermans	08/01/11	07/31/12	27,000 *
The Jane Coffin Childs Memorial Fund for Medical Research	Dr. Kuhn	03/29/10	01/31/12	50,500
CSHL Association Fellowship	New Investigator Support	01/01/11	12/31/11	280,000 *
Fundacion Rafael del Pino (Spain)	Dr. Fernandez-Marco	01/01/11	12/31/11	50,000 *
German Academic Exchange Service	Dr. Bergmann	09/01/11	08/31/12	14,900 *
Genentech Foundation	Dr. Joshua-Tor, Watson School of Biological Sciences	07/01/09	06/30/12	59,384
The Fred C. Gloeckner Foundation, Inc.	J. Calarco	08/01/10	07/31/12	8,100
Lola Goldring	Dr. Stillman	09/01/11	08/31/12	75,000 *
Lita Annenberg Hazen Foundation	Watson School of Biological Sciences	05/01/08	04/30/18	10,000
Human Frontier Science Program	Dr. Canela	05/01/09	04/30/12	53,940
	Dr. Kvitsiani	09/01/08	02/29/12	5,320
	Dr. Skopelitis	04/01/09	03/31/12	53,640
International Rett Syndrome Foundation	Dr. K. Krishnan	10/01/10	09/30/12	50,000
Annette Kade Charitable Trust	Watson School of Biological Sciences	09/01/11	08/31/12	25,000 *
The Esther A. & Joseph Klingenstein Fund Inc.	Dr. Shea	07/01/10	06/30/13	50,000
	Dr. Kepecs	07/01/09	06/30/12	50,000
Susan G. Komen for the Cure	Dr. Anczukow	06/04/09	06/03/12	60,000
Life Sciences Research Foundation	Dr. MacAlister	08/01/11	07/31/14	56,000 *
Topspin Partners	Dr. Premsrirut	01/16/11	02/16/11	41,744 *
NARSAD	Dr. Karakas	01/01/10	12/31/11	30,000
	Dr. Taniguchi	01/01/10	12/31/11	30,000
	Dr. Mirrione	07/15/11	07/14/13	30,000 *
National Cancer Center	Dr. Mao	07/01/10	06/30/12	14,000
Mr. and Mrs. John C. Phelan	Watson School of Biological Sciences	09/01/10	08/31/15	100,000
Marie Robertson Memorial Fund	CSHL Neuroscience Program Support	01/01/11	12/31/11	137,500 *
Sass Foundation for Medical Research Inc.	Dr. Vakoc	11/01/10	10/31/12	161,248
Lauri Strauss Leukemia Foundation	Dr. Vernersson Lindahl	09/01/11	08/31/12	10,000 *
The Swartz Foundation	Dr. Zador	01/01/11	12/31/11	7,500 *
	Drs. Kepecs/Hangya	01/01/11	12/31/11	55,000 *
	Drs. Koulakov/Wei	01/01/11	12/31/11	55,000 *
	Dr. Zador	01/01/11	12/31/11	75,000 *
U.S.–Israel Binational Agricultural Research and Development Fund	Dr. Galon-Wolfenson	10/01/11	09/30/12	45,000 *
Training Support				
Research Foundation of the City University of New York	Undergraduate Research Program	06/01/11	05/31/12	23,000 *
Steamboat Foundation	Undergraduate Research Program	05/01/11	04/30/12	12,000 *
William Townsend Porter Foundation	Undergraduate Research Program	04/01/11	03/31/12	12,000 *

[1]Includes direct and indirect costs
*New or competing renewal grants awarded in 2011

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2011 Funding*[1]	
Course Support					
American Express Foundation	Leadership in Bioscience	11/01/11	10/31/14	$ 75,000	*
Applied Biosystems	Advanced Sequencing Technologies and Applications	07/01/10	06/30/12	30,000	
Coherent	Imaging Structure & Function in the Nervous System	04/01/11	03/31/12	2,500	*
Howard Hughes Medical Institute	Course Support	01/01/07	12/31/11	675,000	
Illumina, Inc.	Advanced Sequencing Technologies and Applications	07/01/10	06/30/12	30,000	
Society for Neuroscience/International Brain Research Organization	Summer Neuroscience Course	07/01/11	06/30/12	16,500	*
Meeting Support					
Amgen USA	76th CSHL Symposium: Metabolism and Disease	02/01/11	01/31/12	2,000	*
Applied Biosystems	Single Cell Analysis	04/01/11	03/31/12	5,000	*
Boston Biochem	The Ubiquitin Family	02/01/11	01/31/12	1,000	*
Bruker AXS Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000	
Bruker Biospin Corporation	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000	
Cambridge Isotope Laboratories, Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000	
Chemical Computing Group Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000	
Enzo Life Sciences, Inc.	The Ubiquitin Family	02/01/11	01/31/12	2,000	*
Genentech Inc.	The Ubiquitin Family	02/01/11	01/31/12	4,000	*
Howard Hughes Medical Institute	Viruses and Oncogenes: Celebrating Stephen Goff	10/01/11	09/30/12	5,600	*
Illumina, Inc.	Single Cell Analysis	04/01/11	03/31/12	3,900	*
The March of Dimes Foundation	Germ Cells	10/01/10	03/31/11	5,000	
Millennium Pharmaceuticals, Inc.	The Ubiquitin Family	06/01/11	05/31/12	10,000	*
Novartis Pharmaceuticals Corp.	The Ubiquitin Family	02/01/11	01/31/12	2,500	*
OSI Pharmaceuticals, Inc.	The Biology of Cancer: Microenvironment, Metastasis, and Therapeutics	02/01/11	01/31/12	25,000	*
Peptide Institute, Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	5,000	
Progenics Pharmaceuticals, Inc.	Viruses and Oncogenes: Celebrating Stephen Goff	10/01/11	09/30/12	1,000	*
Rayonix, L.L.C.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	500	
Rigaku Americas Corporation	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000	
Seattle Biomedical Research Institute	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000	
SGI Japan, LTD	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	3,500	
Toray Industries, Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	2,000	
University of Pennsylvania	Single Cell Analysis	04/01/11	03/31/12	5,000	*
Library Support					
The Ellen Brenner Memorial Fund		12/15/11	12/14/12	2,000	*
National Endowment for the Humanities		02/01/11	07/31/12	6,000	*
National Historical Publications and Records Commission		08/01/11	10/31/12	66,361	*
New York State Education Department		07/01/10	06/30/11	3,701	
Wellcome Trust		08/01/11	07/31/13	298,008	*

[1]Includes direct and indirect costs
*New or competing renewal grants awarded in 2011

DNA LEARNING CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2011 Funding*[†]
FEDERAL GRANTS			
National Institutes of Health	Science Education Partnership Award (SEPA): Nationwide Dissemination of *Inside Cancer* Internet Site	8/08–7/11	$ 37,060
National Science Foundation	Course, Curriculum, and Laboratory Instruction (CCLI) Program: Nationwide Dissemination of RNAi Curriculum	9/07–8/11	44,882
National Science Foundation, University of Arizona	Educational Outreach for *iPlant*: A Cyberinfrastructure for Plant Sciences	2/08–1/13	783,255
National Science Foundation, Cornell University	*Weed to Wonder* Internet Site Development: Educational Outreach for Functional Genomics of the Maize Shoot Apical Meristem	9/08–8/11	51,818
National Science Foundation, North Carolina State University	GEPR: Epigenome Dynamics During DNA Replication	3/11–2/13	68,700
National Science Foundation	Advanced Technology Education (ATE) Program: Genomic Approaches in Biosciences	4/11–3/14	173,883
NONFEDERAL GRANTS			
Victor Centers for Prevention of Jewish Genetic Diseases, Albert Einstein Healthcare Center/ Marcus Jewish Genetic Disease Consortium	*Gene Screen* iPhone/iPad Application	12/10–12/12	39,150
Alfred P. Sloan Foundation	DNA Barcoding Experiments by New York City High School Students (*Urban Barcode Project*)	1/11–7/12	225,466
Hewlett Foundation	*Genes to Cognition Online* Internet Site: Dissemination and Evaluation	10/08–4/11	41,756
Howard Hughes Medical Institute	Pre-College Science Education Initiative: NYC Teacher Professional Development	9/07–8/12	138,875
Dana Foundation	*Harlem DNA Lab* Operating Support	3/09–2/12	124,666
Lounsbery Foundation	Biotechnology Footlocker Program at *Harlem DNA Lab*	11/09–10/11	1,537
William Townsend Porter Foundation	Scholarships for Minority and Underserved Students at *Harlem DNA Lab*	3/10–3/11	17,000
Bank of America	Scholarships for Minority and Underserved Students on Long Island	2011	25,000
National Grid Foundation	Scholarships for Minority and Underserved Students in the Brentwood Union Free School District	10/10–10/11	15,000
Spinal Muscular Atrophy Foundation	*Learn About SMA* Internet site	4/11–4/13	131,000

The following schools and school districts each contributed $1000 or more for participation in the *Curriculum Study* Program:

Bellmore–Merrick Central High School District	$ 1,500	North Shore Central School District	$ 3,000
East Meadow Union Free School District	1,500	North Shore Hebrew Academy High School	1,500
Elwood Union Free School District	1,500	Oceanside Union Free School District	1,500
Garden City Union Free School District	3,000	Oyster Bay–East Norwich Central School District	1,500
Green Vale School	1,500	Plainedge Union Free School District	1,500
Harborfields Central School District	1,500	Plainview–Old Bethpage Central School District	1,500
Herricks Union Free School District	1,500	Portledge School	1,500
Huntington Union Free School District	3,000	Port Washington Union Free School District	1,500
Island Trees Union Free School District	1,500	Ramaz Upper School	3,000
Jericho Union Free School District	3,000	Roslyn Union Free School District	1,500
Levittown Union Free School District	1,500	Sachem Central School District	1,500
Long Beach City School District	1,500	South Huntington Union Free School District	1,500
Manhasset Union Free School District	3,500	Syosset Central School District	1,500
Massapequa Union Free School District	1,500	West Hempstead Union Free School District	1,500
Northport–East Northport Union Free School District	2,000		

The following schools and school districts each contributed $1000 or more for participation in the *Genetics as a Model for Whole Learning* Program:

Bay Shore Union Free School District	$ 4,800	Laurel Hill School, St. James	$ 1,200
Bellmore Union Free School District	1,500	Lawrence Union Free School District	6,700
Bellmore–Merrick Central High School District	23,800	Locust Valley Central School District	8,309
Bethpage Union Free School District	1,600	Merrick Union Free School District	5,600
East Meadow Union Free School District	4,550	MS 447–The Math and Science Exploratory School, NYC	1,500
East Williston Union Free School District	2,800	North Bellmore Union Free School District	1,450
Elwood Union Free School District	3,950	Oceanside Union Free School District	1,500
Floral Park–Bellerose Union Free School District	6,500	Rockville Centre Union Free School District	5,400
Friends Academy, Locust Valley	2,800	Smithtown Central School District	1,400
Garden City Union Free School District	14,975	St. Dominic Elementary School, Oyster Bay	4,050
Half Hollow Hills Central School District	9,150	St. Edward the Confessor School, Syosset	2,025
Hebrew Academy of Five Towns	1,200	St. Patrick School, Huntington	1,350
Herricks Union Free School District	4,050	Syosset Central School District	32,800
Hofstra STEP Program, Hofstra University	1,800	Three Village Central School District	6,000
Huntington Union Free School District	4,650	Trinity Regional School, Northport	1,100
Island Park Union Free School District	2,200	Valley Stream 13 Union Free School District	1,350
Jericho Union Free School District	8,125	Yeshiva Darchei Torah School, Far Rockaway	1,920

[†]Includes direct and indirect costs

BANBURY CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2011 Funding*
FEDERAL SUPPORT			
Defense Threat Reduction Agency Department of Defense	Scientific and Technological Barriers to Global Real-Time Risk Assessment of Vector-Borne Infections	2011	$ 34,929
NIH–National Institute of Mental Health	The 3rd Annual NIMH-Sponsored Brain Camp	2011	41,325
National Science Foundation	The Future of Plant Genome Sequencing and Analysis	2011	34,196
NONFEDERAL SUPPORT			
Boehringer Ingelheim Fonds	Science: Get it across!	2011	65,472
CFIDS Association	Strategic Research Initiative for CFS	2011	33,397
Cold Spring Harbor Laboratory Corporate Sponsor Program	Genotype to Phenotype: Deriving Biological Knowledge from Large Genomic Datasets	2011	42,051
Cold Spring Harbor Laboratory Corporate Sponsor Program	Transmissible Amyloidoses	2011	41,078
Cold Spring Harbor Laboratory Corporate Sponsor Program	Myc and the Pathway to Cancer	2011	45,337
Cold Spring Harbor Laboratory–Pioneer/DuPont Collaborative Research Program	Dosage, Epigenetics, and the Biology of Hybridization and Hybrids	2011	51,155
Epigenomics of Plants International Consortium	Board Meeting	2011	17,384
FEBS Journal	FEBS Journal Editorial Board Meeting	2011	10,020
The Gatsby Charitable Foundation	Neuronal Response Variability and Cortical Computation	2011	15,000
Hazen Polsky Foundation	Curing Melanoma and Other Cancers by Targeted Therapies	2011	50,000
Individual participants	The Future of Biomarker Discovery and Biobanks in Cancer Diagnosis, Prognosis, and Therapy	2011	2,610
Individual participants	Scientific and Technological Barriers to Global Real-Time Risk Assessment of Vector-Borne Infections	2011	15,665
John Wiley & Sons Ltd	FEBS Journal Editorial Board Meeting	2011	1,695
The Lehrman Institute	DNA and the History of Mankind	2011	170,245
Leukemia & Lymphoma Society	SCOR Retreat	2011	7,235
Marie Robertson Research Fund	Evolution of Neural Circuits and Behavior	2011	20,000
Melanoma Research Alliance	Curing Melanoma and Other Cancers by Targeted Therapies	2011	50,000
Oliver Grace Fund	Antibiotic Resistance: Past, Present, Future	2011	46,970
Oliver Grace Cancer Fund	Metformin and Neoplasia	2011	51,121
Sanofi-Aventis TSU-Aging	Translation of Cellular and Molecular Mechanisms of Aging to Geriatric Disorders	2011	44,817
The Swartz Foundation	Neuronal Response Variability and Cortical Computation	2011	43,266
The Stanley Research Foundation	Psychiatric Genomics	2011	63,607
SWOG	The Future of Biomarker Discovery and Biobanks in Cancer Diagnosis, Prognosis and Therapy	2011	30,943
Time for Lyme, Inc.	Lyme Disease in the Proteomics-Genomics Era	2011	41,304

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 2011 as the economic difficulties began to take effect. The year 2012 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 eight representatives of the Corporate Sponsors at our meetings. Three scientists from Sponsors may attend meetings at the Banbury Center, where attendance is otherwise only by invitation of the organizers. Member companies also receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals *Genes & Development, Learning & Memory, Genome Research*, and *RNA*.

We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory website on the Meetings Office and Banbury Center pages. Members in 2011 were the following:

CORPORATE SPONSORS

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DEVELOPMENT

Cold Spring Harbor Laboratory had a noteworthy year in 2011, with many scientific breakthroughs. In June, we published the most comprehensive genetic road map for autism to date. The goal of this work is to design more immediate and accurate diagnostic tools for autism, which will allow for earlier intervention. In August, using advanced genetic tools developed at CSHL, we discovered a new target for a very lethal form of leukemia. Collaborating with the Dana Farber Cancer Institute, we were successful in identifying a drug for this target that will enter clinical trials next year. Using a similar strategy, CSHL researchers are pinpointing new targets for melanoma, pancreas, breast, and prostate cancers. Through these advancements, the Laboratory is moving beyond basic research and is focusing its efforts on developing therapeutics for cancer and neurological diseases. Dr. David Tuveson—a world-renowned scientist who focuses on pancreatic cancer and melanoma—will join the Laboratory in 2012. In collaboration with the Lustgarten Foundation and Memorial Sloan-Kettering Cancer Center, Dr. Tuveson will be leading CSHL's program in cancer therapeutics.

We were deeply saddened by the loss of Honorary Trustee, Double Helix Medal recipient, and Founder of the Breast Cancer Research Foundation (BCRF), Evelyn Lauder. Through her work at BCRF, Evelyn raised millions of dollars for breast cancer research and distributed these funds to breast cancer researchers around the world, including CSHL scientist Mike Wigler. Thanks to BCRF and Evelyn, women and men diagnosed with breast cancer today are recovering and surviving at much higher rates. The Laboratory also lost Peter Stehli, a longtime friend, supporter, and former member of the Development Department. Peter's welcoming and enthusiastic spirit made him an instant friend to everyone he met, and he will be greatly missed.

This year, our Double Helix Medals Dinner was a huge success as we honored three remarkable people: Kareem Abdul-Jabbar, Temple Grandin, and Harold Varmus. In their own unique way, each of the recipients has had a tremendous impact on genetic disease awareness and research. With more than $3 million raised, this event has become an important source of unrestricted funding for the Laboratory.

I would like to take this opportunity to thank all of our dedicated volunteers and loyal supporters. With your help, the Laboratory continues to be rated one of the top research institutions in the world, and our researchers are making significant strides against the devastating diseases that afflict humankind.

Charles V. Prizzi, *Vice President for Development*

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the State of New York. Less than half of the Laboratory's annual revenues are derived from federal grants and contracts, and thus, it relies heavily upon support from the private sector: foundations, corporations, and individuals. CSHL takes this occasion to sincerely thank each and every donor whose generous contributions have made possible the discoveries we report in this publication. There are a variety of ways to give to the Laboratory:

Capital and Endowment Campaign Support: Donations help to secure the financial stability of CSHL and provide resources to expand the facilities and staff.

Research Support: Donations in specific areas of research help to increase the speed at which genetic discoveries are translated into diagnostic tests and therapeutics.

Annual Fund: Donations provide funding for some of the most innovative young researchers in science today and constitute an important investment in groundbreaking research in cancer, neuroscience, plant biology, and bioinformatics.

Science Education: Donations support programs at the DNA Learning Center and the Watson School of Biological Sciences, where the next generation learns about genetics in an exciting and interactive environment.

Planned and Estate Gifts: Individuals who inform us of their intention to make a gift to CSHL from their estate are invited to become members of The Harbor Society. Estate gifts help to ensure that CSHL will continue to pursue its mission for many years to come.

For additional information, please contact Charlie Prizzi, Vice President for Development, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6961. E-mail: prizzi@cshl.edu

President's Council

An outstanding lecture program this year included talks on addiction's numerous manifestations, the physical, emotional, and fiscal toll addiction imposes on individuals and society as a whole, extraordinary advancements in brain-imaging technologies, and the molecular mechanisms that drive addictive behavior. Lara and Remy Trafelet hosted our April reception at their beautiful Park Avenue apartment, where CSHL Assistant Professor Anne Churchland spoke about the role of decision making in addiction.

Former Congressman Patrick Kennedy kicked off the October retreat at the Banbury Conference Center with a powerful talk about his own struggles with addiction and his advocacy for increased national investment in basic and translational neuroscience. Guests were moved by an evocative photo exhibit, *Expressions of Addiction*, by speaker Howard Shaffer, a Harvard psychiatrist. Co-chairs of this year's meeting were CSHL Trustee Howard Morgan and Council members Cynthia Stebbins and Steve Wiggins.

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Dr. Bruce Stillman

Chairpersons

Howard Morgan
Cynthia Stebbins
Steve Wiggins

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Mr. and Mrs. Howard J. Tytel
Dr. and Mrs. James D. Watson
Mr. Roy J. Zuckerberg

Bruce Stillman, Anne Churchland, and Lara and Remy Trafelet

Liz Watson, Patrick Kennedy, Jim Watson, Catherine Moraetis, and Greg and Susan Echt

Cold Spring Harbor Laboratory Association

With 1200 members, the Cold Spring Harbor Laboratory Association (CSHLA) continues to increase awareness of CSHL, and this year helped raise nearly $6 million in unrestricted support for the Laboratory. Events and benefits spearheaded by Directors of the Association included our first Labapalooza fund-raiser featuring local restaurants and artists and music by Lab researchers; the Double Helix Medals Dinner in New York City; a golf tournament at Piping Rock Club; Symposium dinner parties; the Women's Partnership in Science luncheon, and a variety of receptions and dinners that introduced new friends to the exceptionally talented young scientists at CSHL.

Mary Lindsay, former CSHLA President and Honorary Director, continues to set an example for all with her participation at many Laboratory events, commitment to CSHL research and education programs, as well as her interest in the day care center that bears her name. Mary is truly the epitome of what it means to give back to the institutions you believe in.

Special thanks go to Sandy Tytel, Association President, as she enthusiastically led the Association Directors to new levels of participation in all aspects.

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Anne R. Meier
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2011 CSHLA Directors

DNA Learning Center Corporate Advisory Board

The Corporate Advisory Board (CAB) continues to be a vital liaison between Cold Spring Harbor Laboratory (CSHL) and the tristate business community. Established more than 20 years ago, the CAB continues to be the driving force behind the extremely popular CSHL annual golf outing held in June at Piping Rock. Special thanks go to Eddie Chernoff, who not only has been the Chairman of the CAB for more than 10 years, but continues to chair the golf outing as well. On a beautiful June day, over 140 golfers participated in the 18th annual golf tournament, which raised more than $125,000.

Laurie Landeau, CSHL Trustee and liaison to the Corporate Advisory Board, continues to champion the efforts of the DNA Learning Center through her quiet but remarkable support and guidance.

Jack O'Donnell, Judy and Barney Fortunato, and Barney Fortunato, Jr. at CSHL's 2011 Golf Tournament

CAB member Pat Peterson with colleagues from Daniel Gale Sotheby's International Realty at CSHL's 2011 Golf Tournament

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On November 15, the sixth Double Helix Medals Dinner was held at the Mandarin Oriental in Manhattan. CSHL Chancellor Emeritus Jim Watson (*far left*) and President Bruce Stillman (*far right*) appear with 2011 Double Helix Medals Dinner honorees Harold Varmus (*middle left*), Kareem Abdul-Jabbar (*back*), and Temple Grandin.

CSHL Vice Chairman Marilyn Simons with her husband Jim Simons at the 2011 Double Helix Medals Dinner

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CSHL Board of Trustees Chairman Jamie Nicholls with Peter Quick, Tom Quick, and Fran Biondi (*left to right*) at the 2011 Double Helix Medals Dinner

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Meg Braff, Catherine Colley, Elizabeth Ainslie, Emily Johnson, and Lisa Eastman (*left to right*) at the Women's Partnership in Science luncheon held on September 13, 2011 at Cold Spring Harbor Laboratory

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CSHL President Bruce Stillman with Karen Orzel (*left*) and Caroline Monti Saladino, President of the Don Monti Memorial Research Foundation. Photo by Miranda Gatewood; courtesy of *Networking* magazine.

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Carole Alexander, Fiona Whalley, and Elisa Paone of the Manhasset Women's Coalition Against Breast Cancer presented CSHL researcher Dr. Mikala Egeblad (*third from left, front row*) and members of her laboratory with a check to support breast cancer research.

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