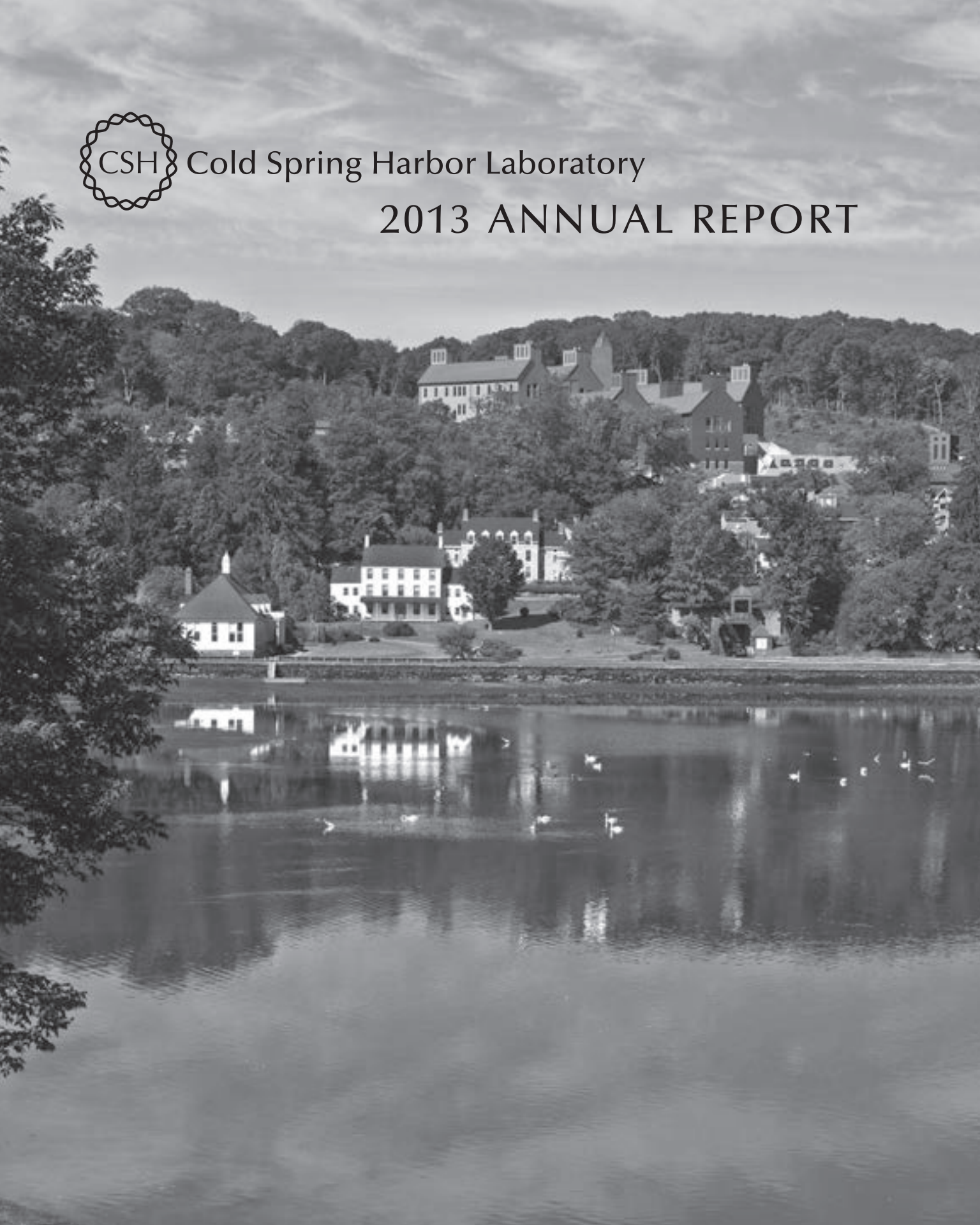
Cold Spring Harbor Laboratory

2013 ANNUAL REPORT



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

www.cshl.edu

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Kathryn Wasserman Davis (1907–2013)

Kathryn Wasserman Davis was 106 when she died—remarkable in her number of years and how she lived every day to the fullest. Her dictum: "I want results in my lifetime." She certainly made things happen. She was best known for passion and philanthropic support of cross-cultural education and international relations, but our institution benefited greatly from Mrs. Davis's deep appreciation for scientific discovery. At CSHL, we remember her fondly for her infinite curiosity and youthful exuberance.

Mrs. Davis once admitted to a journalist that she "always hated science and avoided it like the plague." She explained that this was because "I didn't understand it and no one ever gave me the opportunity to learn." But when given the opportunity to learn from CSHL's experts, she grew to be a champion of biomedical research. Jim Watson and I were fortunate to get to know Mrs. Davis when she was in her 90s, a time when she was exploring new interests including painting, kayaking, and saving the Hudson River Valley. Her remarkable oil paintings hung proudly in her homes and often dominated our conversations over lunch or tea. Kathryn always maintained her leadership role as the widow of one of the United States' great Ambassadors, Shelby Cullom Davis, banker, philanthropist, and US Ambassador to Switzerland from 1969 to 1975.

An undergraduate at Wellesley College, Kathryn later graduated from Columbia University with a Master's and from the University of Geneva with a Ph.D., both in international relations. An avid traveler, she continued her passion for travel even through her later years, visiting many countries and meeting world leaders. In her own right, she was a very special ambassador for the United States.

With encouragement from her daughter Diana, son Shelby, and grandson Christopher, she proved an eager student of some of the most complex and cutting-edge technologies being developed at CSHL. Mrs. Davis became a prized student of RNA interference (RNAi) pioneer and CSHL Professor Greg Hannon, who partnered with Mrs. Davis on a research mission to create a set of tools to silence every gene in humans and mice, search for new therapeutic targets for cancer in the human genome, and implement a genetically based attack on human cancer cells.

Celebrating her 100th, she proudly announced in a birthday letter to friends the founding of the Kathryn W. Davis RNAi Research Program here. Headed by Greg Hannon, this program pursues the understanding and use of RNAi technology. This technology enables researchers to silence genes in cells grown in culture and in animals, allowing for the study of gene functions and of roles in cancer and other diseases. The generosity of Kathryn and her family through the Shelby Cullom Davis Foundation, which was named for her late husband, has enabled scientists throughout the world to use RNAi technology for many diverse experiments.

With two sisters who had suffered from breast cancer, Mrs. Davis was keenly interested in CSHL's multipronged efforts in cancer research. She also spearheaded collaborative efforts between CSHL and the Jackson Laboratory toward finding a cure for glaucoma, a condition that

affected her during her last years. In these research areas and in most everything she pursued, Mrs. Davis said she always wanted to be "ahead of the curve." She took a very personal approach to philanthropy and it was important to her not only to understand the science that we were doing but also to get to know the scientists themselves.

Her friendship with Dr. Hannon, who was honored to serve as the first Davis Chair in Human Genetics at CSHL, was special. He called Mrs. Davis the "energizer bunny." Her friends from CSHL were in full force to present her the 2009 Double Helix Medal for Humanitarianism. She took the stage at the gala that night in New York City to accept the medal and easily stole the hearts of all 400 guests!

I am quite sure that someone like Kathryn Davis comes along but once every 100 years or so. To her family, we express our sympathy. In the words of Jim Watson, who in a March 2005 letter to Diana, wrote, "your mother is an inspiration to us all. Between her 'Navratilova-genes' and 'Monet-genes' she is truly an example of genetics at its best. . . . With your help, we can truly change the paradigm of human genetics research and set in motion the paths to stop genetic disease from holding back the lives of so many of the world's peoples."

Bruce Stillman

Arthur M. Spiro (1925–2013)

As I was writing the president's essay for this Annual Report on the topic of public science education, I remembered Arthur and the deep commitment that he and Joan extended to the Lab's DNA Learning Center (DNALC) programs. I think he would concur with my proposition that what we have created with the DNALC can and should be a model for education across this nation. Thank you, Arthur, for your support.

Arthur served on the CSHL Board of Trustees from 1999 until 2006, where he was active on many committees, including the executive, audit, and Woodbury Genome Research Center committees. But he was most passionate about the DNALC Committee that he chaired from 2001 to 2006. As the trustee liaison to the DNALC and its Corporate Advisory Board, Arthur contributed his business savvy and entrepreneurial thinking to the growth of our educational programs and facilities.

Arthur and Joan introduced CSHL to Clemson University, where in 1945 Arthur earned a B.S. in Chemical and Textile Engineering. Half a century later, he would return to Clemson as an adjunct professor in the College of Business and Behavioral Sciences, Trustee of the University Foundation, member of the President's Advisory Council, and, with Joan at his side, founder of the Arthur M. Spiro Institute for Entrepreneurial Leadership. The mission of the Spiro Institute symbolized Arthur's own meteoric rise in the global textile industry from technician to designer, manufacturer, merchant, and executive manager.

It was his vision that leveraged a scientific collaboration between the CSHL plant biology group and the Clemson Genomics Institute to establish the DNALC at Clemson University in 2006. He and Joan saw this partnership between institutions as a way to enhance public science education in South Carolina schools and beyond. Partnering DNALC teaching methodologies and technologies with university outreach programs has proven very successful. Just this year, we launched another such partnership with the University of Notre Dame.

As a tribute, the 100+ seat auditorium at the Dolan DNA Learning Center facility in Cold Spring Harbor Village is named the Joan and Arthur M. Spiro Auditorium. It was dedicated on September 9, 2003, with the wish that their donation to the DNALC "help provide a better quality of life for our families and future generations." Today, more than 450,000 middle and high school students in the tri-state area have experienced hands-on genetics learning experiences as a result.

Arthur and Joan were world travelers, but he was raised in close-by Belle Harbor, Rockaways, and they were very dedicated to Long Island. Arthur was an honorary trustee of North Shore-Long Island Jewish (NSLIJ) Health System and founding Chairman of the Feinstein Institute for Medical Research. We have him to thank for the formation of the partnership between NSLIJ and

our DNALC that is called DNALC West, a DNALC teaching facility located within the NSLIJ facilities at Lake Success. Both the Clemson DNALC and DNALC West set the stage for our later partnership with the NYC Department of Education and the Harlem DNA Lab located at the John S. Roberts Educational Complex.

2013 was the 25th anniversary year of the DNA Learning Center at CSHL. We have accomplished much on the shoulders of great men like Arthur M. Spiro. Our deepest sympathy and respect go to Joan and her daughters and grandchildren.

Bruce Stillman

Colton Packer Wagner (1919–2013)

Former Cold Spring Harbor Laboratory Trustee Colton P. Wagner died this year at the age of 95. He was born in Seattle and graduated from St. Paul's School with an honorary scholarship to Harvard College, from which he received a B.S. in 1941, magna cum laude, Phi Beta Kappa. A distinguished lawyer with an L.L.B. from Harvard Law School, "Colty" helped found the community-based law firm of Humes & Wagner LLP in 1962. He served on the Board of Trustees from 1971 to 1977, a pivotal period in the contemporary history of this institution.

At that time, the Laboratory was the only major scientific organization whose Board of Trustees was composed almost equally of leaders in the local community and notable scientists. During the early 1970s, the staff of CSHL was approximately 100-strong, operating on a budget of just about $3 million. It was a difficult time financially, and the dedication and support of trustees were crucial.

In the year that Colty retired from the board, Jim Watson declared that "our long period of emergency is over." In the 1977 Annual Report, he wrote:

> For the past fifteen years we have had to work very hard—first to survive when the Carnegie Institute of Washington began the close-down of its Department of Genetics, and then to grow again into a serious body for the advancement of fundamental biology. In the beginning the outcome was in doubt, and only through the efforts of many loyal friends did the Participating Institutions come into existence and join forces with our friends in this community to see that we kept going as an independent body. . . . Now we are completely different.

So much happened during Colty's term of service. In 1971, the Laboratory received its first Cancer Center grant from the National Cancer Institute. To this day, CSHL is one of the nation's leading NCI-designated Cancer Centers.

At the same time, the seeds of our neuroscience research program were being sown. In 1971, summer courses in neurobiology began with a view to complementing them with a year-round program of research. By 1977, the focus had turned to initiating such a program to investigate the way nerve cells grow and form synapses.

During Colty's tenure, much was also accomplished in the physical growth and modernization of the campus. But preservation of the historic and environmental beauty of the Cold Spring Harbor shoreline has always been paramount. In 1973, CSHL seized the opportunity to acquire the Whaler's Cove Marina so as not to "lose the tranquility so very essential to our existence and become part of the commercial-urban spread that now dominates so much of the north shore of Long Island." We are sure that Colty, an avid fisherman who was active in local conservation organizations including the Long Island Chapter of the Nature Conservancy, appreciated the tranquility of the Harbor that this purchase continues to guarantee.

We will be forever grateful to trustees like Colty who helped to navigate this institution through rough and sometimes perilous waters. As a seaman who served for 5 years in the U.S. Navy as the Executive Officer on a Second World War destroyer escort, Colty was particularly qualified for such a mission. He retired from the Navy in 1946 as a Lieutenant Commander. We salute his service to CSHL, community, and country. Our condolences go to his children, grandchildren, and great grandchildren.

Bruce Stillman

PRESIDENT'S REPORT

Cold Spring Harbor Laboratory enjoyed a highly successful year in 2013, despite uncertainty about how a political logjam in Washington, D.C., would impact the ability of our scientists to obtain federal grants. In the 20 years since I was appointed Director of the Laboratory, our faculty has grown from 39 to 52 and our federal grant income has increased more than 2.5-fold. Even so, it is clear that federal funding is not keeping pace with the cost of doing science. Federal grants now cover only about 40% of our $119 million research budget, down from approximately 60% support of the $30 million research budget in 1994. In the past decade, federal R&D funding has decreased by about 22% when corrected for inflation, and the fraction of grant applications funded by the National Institutes of Health (NIH) and the National Science Foundation (NSF) has declined alarmingly, to about one in seven of all applications. In forging a political compromise in early 2014 to fund government operations through 2015, Congress failed to engage in a full-scale budget debate about the role of American science in promoting economic development. Although the success rate of CSHL scientists in obtaining NIH grants is more than twice the national average, the funding situation is increasingly tight. Cultivating new sources of private philanthropy is therefore a high priority.

Uncertainty about funding notwithstanding, laboratories of the principal investigators at CSHL made impressive progress in 2013. Elsewhere in these pages are capsule summaries that demonstrate the breadth of our scientific achievements during the year—in cancer research, research on the brain, and in the genetics of plant development. Basic science discoveries continue to drive advances that are increasingly having an impact on medicine and food production.

As our friends and supporters know, CSHL takes pride not only in research achievements, but also in our extraordinary educational programs. These serve a unique range of target audiences, from professional scientists attending one of the world-famous CSHL Meetings or Courses, to a doctoral candidate matriculating in our Watson School of Biological Sciences, to 5th graders at the DNA Learning Center (DNALC) getting their first hands-on exposure to the tools that scientists use to study DNA. In this Report, I focus on our programs that reach young people.

The DNALC was founded 26 years ago to help children and their parents and teachers "thrive in the genome age." The vision that guided the founders was notably democratic and pragmatic: "We envision a day when all elementary students are exposed to principles of genetics and disease risk, when high school students have the opportunity to do hands-on experiments with DNA, and when all families have access to genetic information that they need to make informed health care choices."

The central achievement of the DNALC program, as developed by David Micklos, Executive Director of the Center since its inception, has been to educate all students, not just those who profess and display at an early age an interest in science. My main purpose here is to propose that, in the coming years, the DNALC's hands-on learning model be emulated and reproduced across the sciences and throughout the nation, to the greatest extent possible. We have a winning formula, and it can do much good if scaled up.

What has worked on Long Island and to date has impacted half a million students can work in every major American city and in outreach programs organized for children at every major American university. The Learning Center concept is one that has legs. The more extensively it reaches across the United States, the better prepared Americans will be to make informed health care choices and compete internationally in science and technology fields.

Scientific investigation is a defining feature of our civilization and a prime basis of our hopes for a better future. There is strong evidence that the American people are well aware of this. Year after year, more than 80% of American adults tell pollsters from the University of Chicago's National

Opinion Research Center that "scientific research that advances the frontiers of knowledge should be supported by the federal government, even if it brings no immediate benefits." But the same set of annual surveys, published in *Science and Engineering Indicators* and available online,[1] reveal that Americans score poorly when asked nine questions about basic scientific facts. Many Americans don't understand what radioactivity is, many don't understand that antibiotics do not target viruses that cause illness, and more than 50% agree when asked if the following statement is true: "Ordinary tomatoes don't contain genes, while genetically modified tomatoes do."

Adult scientific literacy in the United States is far below a level befitting a nation leading the world in scientific research and technological development. If we expect to continue leading the world in these areas, we must be serious about investing in science education for the rising generation. The need is all the more acute when one considers the rapid emergence of new centers of scientific and technological activity, notably in China and other East Asian nations that are devoting an increasing share of their GDP to science and science education while we are reducing our share of GDP that supports research. It is worth mentioning that the government of Singapore, when planning the city-state's economic future, chose medical and biotechnology as new areas of focus and accordingly came to CSHL for permission to license our model of high school scientific education. They now teach as many students each year as we do on Long Island, but since the population of Singapore is 2 million less than Long Island, one can safely assume that every child in Singapore is taught science using DNALC methods.

It is possible that in relative terms, tiny Singapore may benefit more from our education model than we will—unless, that is, American academic and political leaders, as well as leaders in industry, support better hands-on science education throughout the nation. It is ironic that although our public schools, with federal and state encouragement, have made commitments to stressing education in the so-called STEM fields (science, technology, engineering, and math), Congress has not seen fit to increase funding for scientific research or for science education. The advancement of science may be one of our highest national priorities, but support of educational programs remains stuck in neutral as debates over spending priorities are put off year after year.[2]

My enthusiasm for the "hands-on" concept central in all DNALC programs—which can be adapted to work in every major scientific field—is grounded in years of watching it succeed in real-world educational situations right here on Long Island and in our satellite facilities, notably in our Harlem DNA Learning Center in Manhattan. There, we have been able to serve students and teachers across the largest and most complex school district in the United States.

The ability of the DNALC to reach public and private school students in all five boroughs of the City from a single Harlem school demonstrates its scalability. The programs are scalable by design: Dave Micklos and members of his very talented team have devised various labs and modules that are fully compatible with the New York school system's State-mandated curriculum. These modules take students on journeys of discovery that make elements of the curriculum literally come alive before their eyes. For instance, the DNALC's pioneering lab on mitochondrial DNA has enabled tens of thousands of New York students to learn by doing—by sampling some of their own mitochondrial DNA and later learning how to interpret the DNA sequences that these generate. The children learn something about themselves—about their own genetic heritage and the extent to which it is shared, and not shared, with their fellow students, other members of their species, and indeed with distant species. Ostensibly abstract knowledge in this way becomes personally relevant.

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[1]http://www.nsf.gov/statistics/seind14/; see especially "Science and Technology: Public Attitudes and Understanding," Chapter 7.

[2]"Wild Wild Cards Remain After Proposed Reshuffle of STEM Education," *Science*, 19 April 2013, 258–259; "NIH Swears Off Science Education," *Science Insider*, 27 September 2013; "Congress and NIH Don't See Eye to Eye on Science Education," *ibid.*, 28 January 2014.

Another of the DNALC's great successes is in using the hands-on approach to inculcate the single most important take-away skill from any K–12 science class: the ability to understand scientific reasoning. In the same respected national survey I have already cited, 58% of American adults in 2010 failed to demonstrate a basic understanding of scientific inquiry (regarding the use of evidence to test theories and the concept of "controls"). The figure balloons to 77% among those with a high school education or less.

Two years ago, the DNALC introduced an educational program that has worked marvelously to demonstrate that high school students are perfectly capable, and often brilliant, at understanding how to use science to ask a question and how to design an experiment using the scientific method to try to answer it. The new program, called the Urban Barcode Project, or UBP, involves teams of competing students, many from ethnic groups underrepresented in science. Importantly, these students were not cherry-picked from "gifted" classes; quite the opposite, for many, this experience is their first exposure to science. In 2013, 53 teams used DNA barcoding technology to identify living things in the local environment. They discovered 35 DNA sequences that did not match existing data in GenBank, an international database of DNA information. These new sequences were then published to the database with the students as authors. Teams presented their results at the American Museum of Natural History, with the grand prize awarded to students who investigated ant diversity in the Bronx. In 2012, 65 novel DNA sequences were discovered, and winners of the competition proved that many herbal Ginkgo products contained little or no *Ginkgo biloba* DNA—a lesson learned about science as well as marketing practices.

Each of the DNALC programs is scalable. A grant from the Howard Hughes Medical Institute (HHMI) enabled Micklos and his team to train 835 New York City teachers in lab techniques over a 5-year period. With minimal backup, readily provided by teaching mentors at the DNALC, these teachers have gone back to their schools and taught DNALC lab modules, captivated thousands of children with a hands-on approach, and have been able to build upon lab modules with some of the DNALC's prize-winning websites that extend the lessons from the lab setting and deepen student involvement during much longer periods of time. There are 22 such websites now freely available for use by teachers anywhere who want to use them. The DNALC also has devised "DNA Footlocker" kits that can be rented by mail and provide all the needed materials to do any of their current offering of six different lab modules. This is another aspect of the approach that is infinitely scalable, given proper organization and funding.

A small contingent of teaching experts from the DNALC thus has succeeded in markedly enhancing science education in a school district of more than 1 million students. The multiplier effect of each DNALC-trained teacher is hard to measure, but we do know that more than 40% of the 835 teachers trained under the HHMI grant and 133 additional teachers trained to lead UBP projects have subsequently booked field trips to one of our DNALC facilities; others have explicitly indicated that they felt self-sufficient as a result of their DNALC training and could now introduce students to concepts at their own schools, with the help of rented Footlockers. Students who take our labs appear to do better as well: Approximately a full letter-grade improvement was noted in our most recent attempt to document impact of the labs on students' grades.[3]

I want to make a final point about why this model is ready to be replicated across America. The DNALC has solved a problem that over decades has befuddled other innovative developers of science education curricula. Typically, the creation of programs such as the UBP or the mitochondrial DNA lab is supported by an initial government or foundation grant. When the grant expires, the program is left to succeed or fail on its own. Usually, funds dry up and programs are discontinued. The DNALC has had the insight to charge school districts that can afford to pay a nominal amount, typically a few thousand dollars a year, for the services it provides. Rather than

[3]2011 Annual Report, Cold Spring Harbor Laboratory, "DNA Learning Center Executive Director's Report," p. 448.

hiring a teacher with a Ph.D. in biological science, a high school or middle school knows it can rely on our program to reach large numbers of students, at a tiny fraction of the cost of new faculty.

We annually host more than 20,000 students from Long Island schools for laboratory-based field-trips to our Cold Spring Harbor DNALC facility and its satellites, each of which has dedicated lab space manned by our instructors. Ten thousand more are reached in other programs, including DNA summer camps, a major plus—especially for students in resource-poor urban school districts. Separately, we provide training classes for teachers. No school that is unable to pay is prevented from benefiting from our programs. But many can pay a nominal fee, and the revenues generated make the entire DNALC enterprise economically sustainable, year after year.

Other cities, if they are motivated to do so, can use a similar model to kindle and sustain DNALC-like programs in biology, indeed, all of the physical sciences, as well as engineering. In 2013, we opened a new DNALC at the University of Notre Dame in Indiana, and we have started DNALCs in other areas of the United States, Australia, and Europe. We will open a new, larger DNALC in Manhattan and a new center in Suzhou, China in 2015. It is my dream that one day there will be as many science, technology, and engineering Learning Centers across this nation as there are McDonald's restaurants. It is a lot to hope for, but such a program will immediately make an impact, particularly if we have partners to help. The need is real and, in my view, urgent. By the time they graduate high school, we can prepare our young people to know—every student, not just the highly motivated—how scientists think, how they approach and answer problems, and a bit about how the natural world works. The long-term benefit is to allow more of our citizens to form opinions about subjects grounded in science, including those affecting their own health and well-being.

Bruce Stillman, Ph.D., F.R.S.
President and Chief Executive Officer

Highlights of the Year

Research

How Jumping Genes May Contribute to Age-Related Brain Degeneration

Cognitive decline is often obvious to people as they age. However, corresponding changes in the brain, at a molecular level, are not well understood. This year, Associate Professor Joshua Dubnau and his colleagues used the fruit fly brain as a model to offer insight into a possible molecular basis of neurodegeneration. They identified a class of DNA elements, known as transposons or "jumping genes," that become more abundant and active as the brain ages and cognitive function declines. Transposons are short repetitive sequences of DNA that insert themselves into an organism's genome. When active, transposons copy themselves and jump into other locations within the genome. After the initial stages of life, transposons are typically inactive, but Dubnau and his team discovered that as flies age, transposons become more active and move. A protein called Ago2 is important for regulating transposon levels. When Ago2 is mutated in young flies, transposon levels prematurely increase. At the same time, long-term memory declines, mirroring the memory defects seen in much older flies. Dubnau's work suggests that this "transposon storm" may be responsible for age-related neurodegeneration—a hypothesis his lab is actively working to test.

J. Dubnau

Healthy Cells Support Tumors with Signals That Spur Growth

When researchers and clinicians think of targeted cancer drugs, they are generally looking for therapies that will directly attack tumor cells and spare nearby healthy cells. But new research from Assistant Professor Mikala Egeblad and Associate Professor Scott Powers challenges that strategy. They found that tumors rely, at least in part, on multiple signals from healthy cells in the local environment to promote growth. Egeblad, Powers, and their team systematically cataloged the repertoire of interactions between breast cancer cells and a type of healthy cell that is commonly found in breast tissue. They used genomic analysis to identify 42 different factors that are released by normal cells, the majority of them encouraging cancer growth. They found that each factor came with a different set of instructions for the tumor. Looking closely at three of these factors, the team discovered that one promoted survival, another signaled for greater proliferation, and a third increased inflammation and the growth of nearby blood vessels supporting tumor development. The team blocked these signals, alone and in combination, and found that targeting multiple factors did the most to inhibit tumor growth. Their work has broad implications for future cancer drug development. The most effective cancer therapies, the work suggests, would combine two kinds of drugs: those that target tumors and those that block proliferation signals from nearby healthy cells.

M. Egeblad

S. Powers

Natural Mutations Point the Way to Greater Corn Yield

As the global population soars beyond 7 billion, efforts to increase food production have taken on new importance. This year, Professor David Jackson and his colleagues made discoveries that offer insight into how to naturally boost the production of maize, a staple of the world diet. Known commonly as corn kernels, the edible parts of a maize plant are the seeds. These seeds, like all organs in a plant, are derived from a structure known as the meristem. Jackson began with a simple hypothesis: Increasing the size of the meristem could create more area for kernels to develop and thus lead to an increase in the plant's yield. To test this hypothesis, Jackson and his team sought

D. Jackson

to identify genes that control the size of the meristem. They discovered a mutation in one of these genes, known as *FASCIATED EAR2* (*FEA2*), that results in a larger meristem. Plants grown with the *FEA2* mutation produce ears of corn that have a greater number of rows and up to 13% more kernels than their normal counterparts. In other experiments, Jackson and colleagues identified another mutant gene, known as *COMPACT PLANT2* (*CT2*), that also regulates the size of the meristem. The team explored the molecular basis of these mutations and discovered that the *CT2* gene encodes for a protein known as Gα that unexpectedly interacts with FEA2, an unusual type of cell-surface receptor. Detailed understanding of these natural mutations and their impact on plant growth points the way to higher maize yields—good news if we are to meet the planet's ever-growing food needs.

A. Kepecs

Brain Uses Disinhibition to Obtain Extremely Precise Control

There are two major classes of neurons in the cerebral cortex: excitatory and inhibitory. Assistant Professor Adam Kepecs and his team are working to understand how signaling between these neurons shapes mental processes such as cognition and learning. This year, Kepecs and his team identified the role of a special class of inhibitory neurons, known as VIP neurons, in the cortex. In collaboration with CSHL Professor Josh Huang, they used a technique known as optogenetics to specifically stimulate VIP neurons in the mouse brain. They found that when these neurons are active, signaling from certain neurons decreases, whereas the activity of other neurons increases. Upon closer investigation, Kepecs and his colleagues discovered that VIP neurons were inhibiting other inhibitory neurons in the brain, which allowed the excitatory neurons to become more active. This process, known as disinhibition, is something like releasing the brakes on a car without stepping on the gas pedal. Excitatory neurons are not directly activated, which gives the brain highly precise control over cortical processing. Kepecs and his team found that VIP neurons function in the auditory cortex and mediate reward and punishment behaviors. In linking neuronal signaling with a behavior, Kepecs has gained critical insight that will allow him to further explore the complex networks that control cortical function.

J. Huang

First Animal Model of Adult-Onset Motor Neuron Disease, SMA

Spinal muscular atrophy (SMA) is a rare but devastating motor neuron disease. It is caused by a defect in a type of genetic editing, known as splicing. The splicing error occurs in a gene, called *SMN1* (survival of motor neuron 1), that is required for motor neurons to function and lowers the amount of SMN protein in the body. In its most severe form, the disease affects infants and young children; it is the leading genetic cause of childhood mortality. Patients with more SMN protein and, therefore, more mild manifestations of the disease, can live to adulthood, but they develop debilitating handicaps such as losing the ability to walk. Professor Adrian Krainer, an authority on splicing, has made major contributions to the study of the most serious forms of SMA, and he has taken part in work leading to the discovery and testing of a promising drug, currently in Phase-2 clinical trials. Yet, the adult-onset form of SMA has been challenging to research because scientists have lacked a proper animal model. This year, Krainer led a team to develop a mouse model for adult-onset SMA, which faithfully reproduced many of the pathologies seen in patients. His team was particularly interested in understanding how SMN1 protein levels affect neuronal function in adults. They were excited to discover that only moderate levels of the protein are needed, suggesting that clinicians will have a broad time window in which to treat adult patients with SMA.

A. Krainer

Mathematical Technique to Declutter Cancer Cell Data

A. Krasnitz

M. Wigler

In recent years, next-generation sequencing has brought genome-wide data to clinical research, paving the way for major new discoveries in the diagnosis and treatment of disease. Despite this promise, there has been some significant challenges. Sequencing generates extremely large data sets that cannot easily be interpreted. Advanced computing technology is required to extract meaningful information from the data. This year, Assistant Professor Alexander Krasnitz and Professor Michael Wigler developed a mathematical method of simplifying and interpreting genome data based on variations in the sequence. These changes in genomic sequence are common in diseases such as cancer, where certain segments of DNA may be amplified or deleted in a tumor cell. These mutated regions are called "intervals" and may appear repeatedly within a specific population of cells. When the intervals are superimposed against a map of the full human genome, they form "stacks" at discrete locations. Because of the vagaries of collecting genome data and a certain amount of small-scale variation in the precise boundaries of the deleted or amplified DNA intervals, these stacks are wobbly. This makes them very hard to accurately interpret. Krasnitz and Wigler devised a mathematical method called CORE to clean up the stacks of overlapping data, revealing a rich structure underlying all of the clutter. Such analysis is a potentially valuable guide to prognosis and can also help to make important treatment decisions.

Finding Where Fear Memories Form in the Brain

B. Li

Fear elicits a similar response in animals and humans: a momentary feeling of paralysis as the brain assesses present danger. This past year, Assistant Professor Bo Li examined how this universal emotion is learned, controlled, and remembered, and how the brain translates fear into action. Li and his team trained mice to be afraid of an auditory cue. They used a technique called optogenetics to activate specific neurons with laser light, directed fiberoptically into the brain with pinpoint accuracy. They could then record changes in the behavior of the mouse to determine how stimulating a particular area of the brain results in action. Li's team found that fear conditioning alters the release of neurotransmitters in a part of the brain called the central amygdala. The central amygdala is associated with emotion, pain processing, and reward-based behavior. The team further examined this portion of the brain and discovered that both formation and recall of fear memory requires the activation of a specific class of neurons, known as somatostatin-positive neurons, within the central amygdala. This work demonstrates that specific subsets of neurons in the central amygdala have an active role in the brain's fear response, converting fear into action. Li's lab hopes to build on these discoveries to understand the neural circuit changes involved in posttraumatic stress disorder (PTSD).

Genetics Explains Variable Severity of Birth Defects in EEC Syndrome

Cleft palate can be part of a larger condition known as ectodactyly, ectodermal dysplasia, clefting syndrome (EEC), that can be debilitating and sometimes fatal. The illness is caused by a well-known DNA mutation in the *p63* gene, but the birth defects that result range in severity. In fact, not all children with the *p63* mutation develop EEC. This year, Professor Alea Mills and her team discovered a genetic modifier of the *p63* mutation that explains how two people with the same mutation can manifest the illness very differently. They engineered a mouse in which the normal copy of *p63* was replaced with the mutated version seen in EEC. These mice developed with birth defects ranging in severity, similar to babies with EEC. A complex series


A. Mills

of genetic experiments revealed that the presence or absence of one variant type of the p63 protein, called TAp63, determines whether or not a child with the *p63* mutation will actually develop EEC pathology. Loss of TAp63 alone does not cause EEC. But when mice lacking TAp63 also possess the EEC-causing *p63* gene mutation, pathology always occurs. This work suggests that in children who have inherited the EEC-causing mutation from one of their parents, levels of the TAp63 protein determine whether and to what extent these children will be born with birth defects. Mills speculates that when levels of TAp63 drop beneath a certain threshold, it is no longer protective, opening the way to pathology. The next step for researchers in this area is to compare the DNA of children only mildly affected by EEC with siblings or other children who have a severe form of the disease.

Linking Brain Cell Activity with Smell Recognition and Behavior

An orange and a grapefruit have quite similar odors; at the same time, they are both sweet and acidic. Despite the similarity, our brains can readily differentiate between the two. Associate Professor Glenn Turner and colleagues have made significant advancements this year using the fruit fly olfaction system to identify how the brain distinguishes one scent from another. The fruit fly brain has a structure called the mushroom body that is responsible for learning and memory, including olfaction. Within the mushroom body are neurons called Kenyon cells that have unique response properties. Signals from these neurons are rare and often weak. To determine if and how Kenyon cells function in odor memory, Turner and his team used an imaging technique that allowed them to monitor and measure the response of more than 100 Kenyon cells at once. They found that their sparse responses allow these neurons to integrate multiple signals that provide a large amount of information about a particular odor. They discovered that as few as 25 neurons are required to distinguish among similar scents. This information will help Turner's team to better understand how the brain combines sensory data to make decisions.


G. Turner

Growth Factors Contribute to Drug Resistance in Pancreatic Cancer

Pancreatic cancer is one of the most deadly and intractable forms of cancer, with a 5-year survival rate of only 6%. New treatments are urgently needed. This past year, Professor David Tuveson uncovered a new avenue for drug development. It is thought that many existing chemotherapies fail because they are unable to reach the interior of pancreatic tumors. Tuveson and his colleagues tried to address this problem by targeting high amounts of a chemotherapy drug directly into the tumor. Unfortunately, the response did not improve, suggesting that other factors contribute to tumor survival. The team discovered proteins, known as growth factors, that promote tumor growth in the pancreatic tumor mass. Using an antibody against just one of these factors, called connective tissue growth factor (CTGF), the team was able to block nurturing signals in the local tumor environment. When mice with pancreatic cancer were treated with both the antibody and standard chemotherapy, tumor cells died and mice lived longer. Tuveson's work suggests that these growth signals within the tumor overcome the power of conventional chemotherapies. New drugs targeting these survival signals might be used in combination with existing chemotherapies to stop cancer growth.


D. Tuveson

Scientific Advisory Committee

We are honored to receive input from scientific world leaders who serve on CSHL's Scientific Advisory Council (SAC). The SAC is a nine-member Council that also includes participation

from CSHL Trustee James M. Stone, Ph.D., and CSHL Scientific Trustee Michael R. Botchan, Ph.D. Chairing the SAC is Fred Alt, Ph.D., of Harvard University Medical School. Other members include Drs. Cori Bargmann, Joanne Chory, Carol Greider, Leonid Kruglyak, Markus Meister, Kevan Shokat, and Max Wicha.

Technology Transfer


T. Willey

As CSHL's employee numbers and operating budgets have grown significantly in the first decade of the millennium, so have opportunities for commercialization of our research discoveries and technologies. This year, CSHL announced the recruitment of Teri Willey to the new position of Vice President, Business Development and Technology Transfer. Teri brings a wealth of experience from leading transfer and business development for Mount Sinai Medical Center and the founding of several ventures including Cambridge Enterprise Ltd. and ARCH Development Partners.

John Maroney, who for the past 20 years has helped many CSHL investigators launch successful technology start-ups and negotiate licensing agreements, continues in his role as the Laboratory's general counsel. John played a critical part in the establishment of the Broad Hollow Bioscience Park, a collaborative biotech incubator that CSHL helps to lead on the campus of SUNY Farmingdale, the original home of the CSHL spin-off and cancer drug manufacturer OSI Pharmaceuticals Inc. (now owned by Astellas Pharma Inc.).

CSHL is engaged in technology transfer as one way of delivering important discoveries to the public. We are ramping up support to our scientists in partnering with companies, investors, and others to achieve this mission. This support includes identifying science ideal for partnering, finding the best partners, performing good negotiations, managing the corresponding transactions, and developing the team to carry out this work. 2013 was a year to take stock and initiate program changes in order to move forward with a new approach. 2014 will be a year for evolving the program, building the team, and completing new deals with industry.

Cold Spring Harbor Laboratory Board of Trustees

The CSHL Board of Trustees elects members whose academic and professional accomplishments reach beyond the boundaries of science, providing well-informed governance in an increasingly complex fiscal and regulatory environment. The Board welcomed four new trustees: Cornelia I. Bargmann, Ph.D., Jeffrey E. Kelter, Robert W. Lourie, Ph.D., and Thomas A. Saunders III.

The Board congratulated two retiring members, Scientific Trustee Dr. David Botstein and Alan Seligson. Dr. Botstein was the 2013 recipient of the Breakthrough Prize in Life Sciences and donated $100,000 of his prize to CSHL to support its advanced technology courses. Mr. Seligson, who was named an Honorary Trustee, established the Andrew Seligson Clinical Fellowship in


C.I. Bargmann


J.E. Kelter


R.W. Lourie


T.A. Saunders III

memory of his son who died of cancer, helping to set the stage for CSHL's Cancer Therapeutics Initiative.

The entire community mourned the passing of trustees and friends Colton Packer Wagner, Arthur M. Spiro, and Kathryn W. Davis.

Development

Together, the Board, the CSHL Association, the Corporate Advisory Board, and many individual foundations and donors raised more than $6.9 million in unrestricted funds for research and education programs. Thanks to all those who contributed, especially the Charitable Lead Annuity Trust under the Will of Louis Feil and The Simons Foundation for new major gifts.

CSHL is grateful to the Lustgarten Foundation and Roy Zuckerberg for supporting the Cancer Therapeutics Initiative (CTI) and its leaders, including Dr. David Tuveson, an M.D./Ph.D. expert in pancreatic cancer. Infrastructure for the CTI is essential, and with help from David Koch, New York State Empire State Development, and an anonymous donor, construction of the Preclinical Experimental Therapeutics Facility began in November. The project is moving forward on the strength of a $2 million award from Governor Andrew M. Cuomo's Regional Council initiative.

The Governor praised the initiative. "This expansion project demonstrates how Long Island is becoming a leading hub for scientific and medical study," Cuomo said. "With the support of the Regional Council Initiative, CSHL is moving forward with a facility that will enable critical research seeking to advance the quality of healthcare around the globe. I am pleased that the project is now under way and look forward to seeing its positive impact on the region for years to come."

Long Island Regional Economic Development Council Cochairs Kevin Law, President, Long Island Association, and Stuart Rabinowitz, President, Hofstra University, said, "This groundbreaking is a major milestone not only for Cold Spring Harbor Laboratory and Long Island, but also for the critical research CSHL conducts to find new and innovative ways to treat cancer and improve the quality of life for people around the world."

With dedicated state-of-the-art facilities and staff, CSHL will accelerate research aimed at developing new therapeutics for cancer and neurological disorders. Other significant contributors included the estate of Robert B. Gardner, Jr., Lisa Lourie and Dr. Robert Lourie, and the St. Giles Foundation.

CSHL Association Board of Directors

Groundbreaking of Preclinical Experimental Therapeutics facility

CSHL is extending the reach of its science education programs to urban centers all over the world. A new flagship center in Manhattan will serve as the nucleus for DNA learning in New York City. Middle and high school students will have access to hands-on DNA laboratory experiences to gain a greater understanding of their own uniqueness, the implications of personalized medicine, and their shared genetic heritage in America's melting pot. The NYC center has been made possible by a lead contribution by CSHL Trustee Laurie L. Landeau and significant gifts by the Thompson Family Foundation and the Alfred P. Sloan Foundation.

Unveiled this year was a commemorative wall honoring the major donors, who, under the leadership of Board Chairman Eduardo Mestre, contributed to the Hillside Campaign of 2003–2008. This campaign made possible the 2009 opening of six new Hillside Laboratory buildings, representing the largest expansion in the history of the Laboratory. With these research buildings, CSHL increased its research capacity by 40%.

Supported by our trustees, fund-raising events including the eighth Double Helix Medals Dinner (DHMD) in Manhattan opened CSHL's doors to many new friends in the New York metropolitan area. The DHMD honors individuals who have raised public awareness of the significance of biomedical and genetics research. We saluted Robin Roberts for her courage and tenacity in using her celebrity to share information about breast and blood cancers.

Commemorative wall, Hillside campus

Double Helix Medal winners P. Neufeld, R. Roberts, and Barry Scheck with Bruce Stillman

This year's DHMD also highlighted the impact that CSHL has had in leveraging biomedical technology for applications to benefit society. Since 1992, the Innocence Project has used DNA technology to generate evidence leading to the exoneration of 312 wrongfully convicted individuals. This is a concept that cofounders (and 2013 DHMD medal winners) Peter Neufeld and Barry Scheck advanced after attending a conference on the subject organized at CSHL's Banbury Center in 1989. The Banbury Center's role as a think tank bringing science and public policy experts together is unrivaled.

Research Faculty

Awards

- Professor and Howard Hughes Medical Institute Investigator Gregory Hannon won a MERIT (Method to Extend Research in Time) Award from the National Institute of General Medical Sciences. This prestigious award recognizes highly productive scientists by extending funding for an existing research project grant. A leader in the field of small RNA biology, Hannon has sought to understand the biological roles of small RNAs and the underlying mechanisms through which they operate.
- Professor Partha Mitra, Crick-Clay Professor of Biomathematics, was honored with two awards: the George S. Axelby Outstanding Paper Award of the Control Systems Society of the Institute of Electrical and Electronics Engineers (IEEE) and an INSPIRE (Integrated NSF Support Promoting Interdisciplinary Research and Education) grant from the National Science Foundation (NSF). The INSPIRE grant was established in 2012 to address some of the most complicated and pressing scientific problems that lie at the intersections of traditional disciplines. Mitra is weaving together theoretical threads from physics and engineering to arrive at a coherent theoretical framework for understanding connectivity and dynamical behavior of circuits in the mammalian brain. He is applying insights from his theoretical work to the Mouse Brain Architecture Project, with the goal of generating brain-wide maps of interregional neural connectivity.
- Associate Professor Adam Kepecs received the Memory & Cognitive Disorders Award from the McKnight Endowment Fund for Neuroscience, which provides support to encourage breakthroughs in understanding complex systems involved in neuron signaling.
- Assistant Professor Hongwu Zheng was the recipient of the Distinguished Scientist Award from the Sontag Foundation. He is investigating how brain tumors known as malignant glioma emerge from normal neuronal cells and transform into metastatic tumors.
- Four CSHL postdoctoral neuroscientists won NARSAD Young Investigator Awards from the Brain and Behavior Research Foundation. This support is intended to facilitate the transition for young scientists to ultimately work in laboratories that they themselves direct. Three of the CSHL awardees are studying autism spectrum disorder: Guy Horev, Yongsoo Kim, and Keerthi Krishnan. Sandra Aherns is conducting research on schizophrenia.
- Cited for her postdoctoral work in genetics and genomics, Research Investigator Emily Hodges was named one of five finalists in the annual Regional Blavatnik Award for Young Scientists.
- Postdoctoral Fellow Christine Iok In Chio was named a Damon Runyon Fellow in cancer research. As part of the Lustgarten Foundation Pancreatic Cancer Research Laboratory at CSHL, she is evaluating the biological role of oxidative stress in pancreatic cancer.

New Faculty


L. Zhang

Welcome to our new CSHL Fellow Lingbo Zhang, Ph.D., who earned his doctorate in 2013 in a joint program of Massachusetts Institute of Technology and the National University of Singapore. CSHL Fellows direct their own research programs under the guidance of a senior faculty member. They have their own laboratory space and technician, as well as access to all of the Laboratory's core facilities. Fellowship appointments are for 3 years. Dr. Zhang is interested in understanding the self-renewal mechanism of stem and progenitor cells in the blood-forming system, work he hopes will lead to better treatments for a broad spectrum of unresponsive anemias associated with certain bone marrow failure disorders, myelodysplastic syndrome, and kidney disease. His work can also be applied to leukemic cells, a population of malignant cells whose self-renewal machinery has been hijacked.

Promotions


A. Gann

On January 1, 2013, Alex Gann began his appointment as Dean of the Watson School of Biological Sciences. Congratulations to Adam Kepecs, Bo Li, and Zachary Lippman, who were promoted to Associate Professor. Michael Ronemus was promoted to Research Assistant Professor.

Departures

Professor Lincoln Stein is now Program Director, Informatics and Biocomputing at the Ontario Institute for Cancer Research (OICR).

Education Programs


Z. Lippman

Banbury Center

In its 36th year, the Laboratory's think tank for science and public policy known as the Banbury Center was an exceptionally busy place. A total of 23 meetings were held, inviting more than 700 participants from 40 states and 20 countries. Approximately one-fifth of the attendees were from overseas and about one-third were women.

One of the year's highlights was a meeting called *Redesigning Photosynthesis: Identifying Opportunities and Novel Ideas* that brought plant biologists together to consider whether the efficiency of solar energy capture by plants might be improved. A fine example of a meeting on an "emerging topic"—something for which Banbury is known—convened a distinguished group to discuss whether transposable genetic elements might be active during normal neurogenesis, the process by which new neurons are generated. Until now, "jumping genes" have been known for their role in causing pathology.


M. Ronemus

A 2010 WSBS graduate, Yaniv Erlich, now on the faculty of the Whitehead Institute, organized a Banbury meeting to discuss strategies for maintaining privacy of data gleaned from genomes and other bioscience data sets. *Accelerate Genomic Research with Privacy Protections* included participants across disciplines from science to cryptography to ethics.

The Banbury Center also hosted three Watson School of Biological Sciences (WSBS) courses and six courses conducted as part of the Laboratory's Meetings & Courses Program. The Center appreciates support from numerous corporate sponsors and underwriters who helped to make its rich offerings possible.

DNA Learning Center

This past year, some 20,960 students made field trip visits to DNA Learning Center (DNALC) facilities in Cold Spring Harbor, Lake Success, and Harlem. An additional 10,200 students were reached through in-school instruction by DNALC staff. There were also 1640 in-school lab exposures via mobile "Footlocker Kits" used by teachers in their own schools. These teachers previously received specialized DNALC training.

During the summer, 60 week-long biology and genetics summer camps were held in Cold Spring Harbor and eight other locations in New York, Massachusetts, and Connecticut, drawing a record 1240 students. Monthly *Saturday DNA!* sessions drew hundreds more children, parents, and grandparents.

At its main facility in Cold Spring Harbor, the DNALC this year updated museum space that currently features an exhibit on "Our Common Human Origins." More than 100 classes per year take an instructor-guided tour of the museum, which is also open to the public.

In its second year, the Center's innovative *Urban Barcode Project* (*UBP*) scored major successes, with teams of motivated NYC high school students competing, many from ethnic groups underrepresented in science. Fifty-three teams comprising 144 students were accepted into the competition and completed field work, wet labs, and bioinformatics analyses with the support of their teachers and *UBP* staff at *Open Labs* at *Harlem DNA Lab*. Teams presented their results at symposia at the American Museum of Natural History, with the grand prize awarded to Hostos-Lincoln Academy of Science students Kavita Bhikhi and Hillary Ramirez, who investigated ant diversity in the Bronx.

The *BioMedia* team of the DNALC is award winning and trend setting. Their *3D Brain* iPhone app, launched in 2009 and available on multiple platforms, has been downloaded more than 2.2 million times, and it is used not only by teachers and students, as intended, but also by health professionals, patients, and the public. A 2013 iOS update provides current information about 29 primary structures of the human brain.

This year, the BioMedia team introduced live streaming and webinar programs, broadcast from the DNALC's Laurie J. Landeau Multimedia Studio. Altogether, approximately 4.9 million visits were recorded to the DNALC's 22 websites in 2013. DNALC-produced *YouTube* videos drew more than 823,000 views, with apps downloaded 579,163 times. Total visitation therefore numbered a record 6.26 million.

Since 1995, more than a dozen programs worldwide have been modeled directly on the DNALC experience. These centers, across America and in Europe, Asia, and Australia, provide hands-on experiments to more than 150,000 students annually. The DNALC is the largest provider of teacher training in molecular and genomic biology, and it is the only institution capable of offering high-quality biochemical and bioinformatics instruction anywhere in the world. In the fall, a dedication ceremony marked the opening of the latest DNA Learning Center at Notre Dame University. The DNALC also continued to develop its relationship with Beijing No. 166 High School in China.

DNA Learning Center dedication, Notre Dame University

Plans to develop a new full-service DNALC teaching facility in Manhattan received a jump start with the receipt of a $3 million start-up grant from the Alfred P. Sloan Foundation and a $10 million endowment grant from the Thompson Family Foundation. These add to a lead endowment grant of $6 million from CSHL trustee Laurie J. Landeau.

Meetings & Courses Program

With its beginnings in the first annual meeting, in 1933, of the Cold Spring Harbor Symposium on Quantitative Biology—a scientific conference series still going strong—the Meetings & Courses program in the last year attracted more than 11,600 participants. This included upwards of 7200 individuals who attended scientific meetings and more than 1300 trainees, teaching, and support faculty who took part in courses. An additional 3000 scientists from the Asia/Pacific region attended 17 conferences and one summer school held by the Cold Spring Harbor Asia program in Suzhou, China.

78th CSH Symposium

A major feature of CSHL meetings is that there are very few invited speakers. Meeting organizers select talks from abstracts submitted in advance, ensuring that the latest findings are presented and that young scientists have a chance to describe their work. Several meetings are held annually, including *The Biology of Genomes* and *Retroviruses*, but the majority of meetings convene every other year.

A total of 27 academic meetings were held in 2013. The 78th session of the historic Symposium series addressed Immunity and Tolerance and attracted almost 400 participants. The year saw the introduction of several successful new meetings: *Wiring the Brain*, *Metabolic Signaling and Disease,* and *Behavior and Neurogenetics of Nonhuman Primates*. Two special meetings, *Genes & Diagnostics: A Myriad of Issues in Biotech IP* and the *History of Restriction Enzymes*, drew rather different audiences of lawyers and historians, respectively.

The Courses program covers a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim is to teach professional scientists the latest advances that can be immediately applied to their own research. Increasingly, many courses feature a strong computational component as biology grows ever more interdisciplinary, incorporating methodologies from computer science, physics, and mathematics. Instructors, lecturers, and assistants come from universities, medical schools, research institutes, and companies around the world to teach at CSHL.

Grants from a variety of sources support the courses, with core funding provided by the Howard Hughes Medical Institute. The courses are further supported by awards from the National Institutes of Health and the National Science Foundation, private foundations, and other sources. Equipment and reagents are loaned or donated by companies, ensuring that the courses offer training in the latest technologies.

Cold Spring Harbor Laboratory Press

CSHL Press published a single volume in 1933, based on the Laboratory's first Symposium on Quantitative Biology. Since then, the Press has developed a program consisting of seven successful journals, 190 books, and two online services. It is now a digital publisher with a capacity for print production on demand, and in recent years, its staffing, skills, and organizational structure have been reshaped to this end.

In the United States and Europe, budgetary restrictions continued to squeeze research departments and the academic libraries that are the most important purchasers of scientific information. The Press weathered these pressures by maintaining first-class editorial standards and finding fresh ways of promoting its publications to scientists and their institutions worldwide. Examples include three review journals launched in the past 6 years and growth of subscriptions in emerging scientific communities abroad.

Widely cited impact-factor measurements place two Press journals, *Genes & Development* and *Genome Research*, in the top 1% of the 8000 journals in the *Science Citation Index*. Online usage of

these and their sibling journals continues to climb, exceeding 12 million full-text article downloads in 2013, an increase of 25%. *Cold Spring Harbor Protocols* and *Learning & Memory* continued to gain ground. *Cold Spring Harbor Perspectives in Biology* had particularly sharp growth, a vote of approval for its fresh approach of melding journal and book publishing. Testament to its editorial quality was the award of the Nobel Prize in 2013 to three scientists (Drs. Thomas Südhof, Randy Schekman, and James Rothman) who have edited recent *Perspectives* titles.

bioRχiv.org website

The Press published 18 new book titles in 2013. Several were new editions of past best-sellers, including *Antibodies: A Laboratory Manual* and *Lab Math: A Handbook of Measurements, Calculations, and Other Quantitative Skills for Use at the Bench*. Eleven books were generated from the two online *Perspectives* review journals.

In November, bioRχiv, a new preprint service for the life sciences, was launched to permit distribution of scientific manuscripts not yet published in peer-reviewed journals. This enables researchers to establish priority for their work and to benefit from critiques offered by readers as manuscripts are honed for conventional publication in a peer-reviewed journal. bioRχiv provides an exciting platform on which to build other valuable services in future.

Watson School of Biological Sciences

Under the leadership of its new Dean, Dr. Alexander Gann, the Laboratory's Ph.D.-granting program welcomed its 15th incoming class and graduated its 10th. Eight WSBS students were awarded Ph.D. degrees, bringing the total since the school's inception to 66.

Honorary degrees were conferred upon Jack E. Dixon, Ph.D., Howard Hughes Medical Institute Vice President and Chief Scientific Officer and Professor at the University of California, San Diego; and Brigid L.M. Hogan, Ph.D., F.R.S., Professor and Chair of the Department of Cell Biology at Duke University.

2013 WSBS graduates and honorary degree recipients

The 19th Annual Gavin Borden Visiting Fellow Lecture, reception, and dinner were held on October 28. Sean Carroll, Ph.D., delivered the lecture, titled "Brave Genius: A Scientist's Journey from the French Resistance to the Nobel Prize." Dr. Carroll leads the Department of Science Education of the Howard Hughes Medical Institute, the largest private supporter of science education activities in the United States, and is the Allan Wilson Professor of Molecular Biology and Genetics at the University of Wisconsin.

In August, the WSBS welcomed 10 new students. Members of the Class of 2013 were selected from among 274 applicants and represent the United States, Bulgaria, India, Israel, Italy, and Mexico. Other new graduate students entered as visitors from other institutions, including seven from Long Island's Stony Brook University; other current visitors hail from more distant institutions, including several in Germany, France, and Spain.

The achievements of the graduate program continued to grow. Scientific papers published by students appeared in major journals, bringing the cumulative total of WSBS student publications to more than 230. Current and former students won prestigious and highly competitive scholarships and fellowships. Watson School students continued to graduate considerably faster than students at comparable institutions and demonstrated an ability to secure excellent jobs. Fifteen WSBS graduates have thus far secured tenure-track faculty positions.

From June through August, 25 undergraduates from across the United States, as well as from Canada, China, France, India, Nepal, and the United Kingdom, arrived at CSHL to take part in the historic Undergraduate Research Program. These "URPs" (chosen from among 738 applicants!) had the remarkable opportunity to perform advanced research in the laboratory of a CSHL faculty member. This immersive experience reaped intellectual as well as social rewards for the lucky participants, as in past years. The URP Program, along with the equally innovative and competitive Partners for the Future Program, which brings gifted local high school students to CSHL labs for hands-on research experience, is managed by the Watson School.

Members of the 54th Undergraduate Research Program

Library & Archives

The Institutional Repository is a new on-line resource for publications from CSHL researchers. The repository was initiated this year, with the goal of providing the public with direct access to CSHL discoveries throughout its history. Through this repository, anyone can access these scholarly materials. As an initiative of the Genentech Center for the History of Molecular Biology and Biotechnology at CSHL, Robert Wargas and Mila Pollock examined the history of CSHL's initial involvement in the Biotechnology revolution during the late 1970s/early 1980s, as well as the Lab's continuing relationship with the Biotechnology industry.

The Library hosted *The History of Restriction Enzymes* October 19–21, 2013. Attended by more than 150 scientists, history scholars, authors, educators, and students, this was

Institutional Repository for CSHL authors

the fifth meeting in the Genentech Center series on the history of science. Herb Boyer, Stu Linn, and Rich Roberts, pioneers in the field of restriction enzymes, were co-organizers, bringing together scientists involved in the discoveries and research on restriction enzymes dating back to the 1950s and covering developments to the present time.

The Archives participated in several events, displaying elements from various CSHL collections:

- **Alfred Day Hershey Collection.** Materials from the Alfred Day Hershey Collection were displayed at the Scottish Parliament in an exhibit about the philanthropic legacy of Andrew Carnegie, on display from 14 October 2013 until 25 January 2014.
- **Extraordinary Women in Science & Medicine.** A landmark public exhibition, *Extraordinary Women in Science & Medicine: Four Centuries of Achievement*, held at the prestigious Grolier Club in New York City from September 19 to November 13, 2013, showed materials from the Barbara McClintock Collection, including photos, landmark papers, and a corn cob from the 1960s loaned by CSHL Professor Rob Martienssen.
- **From Base Pair to Body Plan: Celebrating 60 years of DNA.** A display of more than 200 images related to the Laboratory's history and its science was a central part of the meeting *From Base Pair to Body Plan: Celebrating 60 Years of DNA*, held at CSHL from February 28 to March 3, 2013. This display stayed on exhibit for the entire 2013 meetings season.

CSHL completed the digitization of the archives of James D. Watson and Sydney Brenner, as part of the Wellcome Digital Library Pilot Project "Codebreakers: Makers of Modern Genetics." The result of this multiyear collaborative effort with Churchill Archives Center Cambridge, the University of Glasgow, King's College London, and University College London is free public access to these and other historic collections from an online portal at www.wellcomelibrary.org.

Infrastructure

Woodbury Genome Center Addition

The year 2013 saw the groundbreaking of a 7000-square-foot addition to the Woodbury Genome Center. This expansion of the Genome Center Animal Facility—the Preclinical Experimental Therapeutics Facility—will serve as a vital core facility supporting the Laboratory's Cancer Therapeutics Initiative. The facility will house a number of cancer diagnostic and therapeutic resources. Construction is expected to be completed by the end of 2014.

Woodbury Genome Center addition (proposed)

Renovated Cairns building

Cairns Laboratory Renovation

The Cairns Laboratory was reconstructed during 2013. The building was originally used as a sheep shed in the early 20th century and was most recently used as a microscopy laboratory. The completion of the new Hershey Building allowed for relocation of microscopy resources and renovation of the Cairns Laboratory. The roof was raised to make way for improved ventilation systems and the building's infrastructure was brought up to modern laboratory standards. The building will be ready for occupancy in 2014 and will house an RNAi Shared Research Resource facility.

Employees at new Syosset Campus

Yellow House Reconstruction: Historic Perspective

The Yellow House was a circa 1830 residence in considerable disrepair. The house had significant foundation and structural issues and had suffered a number of unsympathetic renovations and additions during its lifetime. Deemed uninhabitable, it was demolished and replaced with a new structure.

The Laboratory spent nearly a year working with the State Historic Preservation Officer to obtain permission to demolish and replace the house. Prior to demolition, the Laboratory photographed and documented the entire house, and the records were entered into the Laboratory and New York State archives. A new Yellow House will be used for faculty housing and be ready for occupancy in the spring of 2014.

Syosset Campus

The Laboratory expanded the use of the Syosset Campus in 2013 with the relocation of the Accounting Department from the Nichols Building and the Procurement Department from the Woodbury Genome Center to new offices on the Syosset Campus.

Information Technology

Completed this year was a campus-wide WiFi upgrade that included replacement of aging infrastructure to provide better coverage and speed and greatly improved handling of our high-density venues, notably the Grace auditorium. This project will continue in 2014, providing a further substantial increase in bandwidth.

Information is at the core of our scientific activities. Our ability to collect, manage, and safeguard large volumes of data is critical. Centrally managed CSHL data storage, currently in excess of 3 petabytes, is expected to grow significantly over the coming years. Enterprise-grade data storage of this scale is tremendously expensive to procure and maintain, and the adoption of a financially sustainable data-handling model is essential. To mitigate escalating storage costs, CSHL adopted a new solution, and we are now well positioned to handle current and emerging data storage needs. With the latest expansion, the CSHL system already has a 900-TB disk capacity that can scale up to 21 petabytes.

Additional upgrades were performed to the core and data center distribution layer network for the Hillside and Grace datacenters. Hardware selection was completed, and vendor selection began. To lower maintenance costs and retire aging hardware, we have increased our use of virtual servers.

Community Outreach

CSHL Public Lectures

February 13–Dr. Richard E. Leakey, Professor, Chair, Turkana Basin Institute; Stony Brook University, Department of Anthropology. *Hominid Evolution: How It Has Shaped Human Behavior, Ethics, and Morality.* 2012 Lorraine Grace lectureship on societal issues of biomedical research (rescheduled from 2012 due to Hurricane Sandy).

June 8–Paul A. Offit, M.D., Chief, Section of Infectious Diseases and Director, Vaccine Education Center at Children's Hospital of Philadelphia; Maurice R. Hilleman Professor of Vaccinology, Professor of Pediatrics at University of Pennsylvania School of Medicine. *Alternative Medicine: Sense and Nonsense.* 2013 Lorraine Grace lectureship on societal issues of biomedical research.

June 25–Josh Dubnau, Ph.D., CSHL Associate Professor; **Peter Davies, Ph.D.**, Head and Scientific Director, Litwin-Zucker Research Center for the Study of Alzheimer's Disease and Memory Disorders, Feinstein Institute for Medical Research; **Jill Goldman, M.S., M.Phil.**, Certified Genetic Counselor, The Center for Parkinson's Disease and Other Movement Disorders, Columbia University Medical Center. *Untangling Dementia–Latest Research and Treatments*, cosponsored by CSHL; U.S. Trust–Bank of America; North Shore–LIJ; and St. Johnland Nursing Center.

July 16–Zachary Lippman, Ph.D., CSHL Associate Professor. *Flower Power and the DNA of Feeding the World*, hosted by The Secret Science Club, Brooklyn, New York.

July 17–Kevin J. Mitchell, Ph.D., Associate Professor of Neurogenetics, Trinity College Dublin, Ireland. *The Miswired Brain—How Altered Brain Development Leads to Mental Disorders*.

August 20–Anne Churchland, Ph.D., CSHL Assistant Professor; *Swinging synapses and decision making*, hosted by The Secret Science Club, Brooklyn, NY.

October 7–Rob Martienssen, Ph.D., CSHL Professor. *Oil Palm and the Rainforest: Genome Sequencing for Sustainability.* Public lecture, Grace Auditorium.

CSHL Public Concerts

April 19:	Ching-Yun Hu, Piano
April 26:	Ying Fang with Ken Noda, Soprano with piano
May 3:	DZ4 with David Kaplan, Woodwind quartet with piano
May 17:	Hye-Jim Kim, Violin (w/piano)
August 23:	Southampton Festival Chamber Orchestra
Sept 12:	Andrew Tyson, Piano
October 4:	Paul Huang, Violin (w/piano)
October 11:	Mikhail Yanovitsky and Galina Sakhnovskaya, Piano and soprano

With help from enthusiastic graduate students and postdoctoral fellows who staff CSHL's public tour program, we conducted 50 campus walking tours throughout the year serving more than 800 visitors. Our guests came from near and wide, including Germany, Italy, and China.

On March 23, more than 400 neighbors came to the main campus for an Open House. Visitors, including many who had never been to CSHL before, plainly enjoyed learning about the spectrum of CSHL research and education programs, as well as ways in which they can be

Open House 2013 (*Top left, right,* and *bottom left*)

J. Ipsarro giving a short science talk

involved, as students, friends, and neighbors. During a continuously running series of 5-Minute Science Talks, CSHL postdoctoral researchers engaged audiences throughout the afternoon on topics from "Why haven't we cured cancer?" to "Molecular photography." Videos of these talks are available on YouTube. Free minitours of the campus led by graduate students and Lab postdocs were extremely popular. Kids and adults alike crammed a booth where DNA Learning Center teachers led hands-on biology demonstrations, featuring DNA extraction.

Local families enjoyed the DNA Day Scavenger Hunt while exploring the history of Cold Spring Harbor Village on April 20. Local institutions including the Cold Spring Harbor Library, the Cold Spring Harbor Whaling Museum, the Firehouse Museum, and CSHL's DNA Learning Center 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. The day commemorates the completion of the Human Genome Project in April 2003 and the discovery of DNA's double helix.

In April, first graders from Goosehill Primary and Friends Academy participated in a science fair. At six stations, they learned about various scientific principles (from chromatography to cell structure to brain anatomy) through hands-on activities and instruction conceived, planned, and led by graduate students and DNALC instructors; 160 students, 14 teachers, and 120 parents participated during the 2 days.

DNALC's Jason Williams led a team of CSHL graduate student and postdoc ambassadors in two iPad app demonstrations at the June 2nd World Science Festival Street Fair in Manhattan's Washington Square Park. CSHL's ambassadors interacted with Festival participants, demonstrating how to use the DNALC-developed apps, 3D Brain, and Gene Screen.

CSHL was pleased to participate in the 10th Long Island 2-Day Walk to Fight Breast Cancer, in which nearly 400 walkers and an equal number of volunteers raised more than $550,000. Since

Long Island 2-Day Walk

R. Sordella (*center right*) participated in Swim Across America (SAA)

the Walk started in 2004, CSHL has received more than a quarter of a million dollars for breast cancer research from LI2DAY.

This year, CSHL Associate Professor Raffaella Sordella joined in the Swim Across America (SAA) "Sound to the Cove Swim" at Morgan Park in Glen Cove. Dr. Sordella received $70,000 from SAA for her research aimed at finding ways to overcome resistance to targeted therapies for non-small-cell lung cancer. Dr. Sordella credited the support she has received from SAA—$420,000 to date—with providing critical resources to identify a population of cells in lung tumors that are intrinsically resistant to therapy.

CSHL Chief Operating Officer Dill Ayres participated in the October 24 *Long Island Press* "The Future of Healthcare on Long Island" summit that brought research institutions, health care providers, and vendors together to highlight the region's assets and challenges.

A member of the New York Academy of Sciences (NYAS), CSHL was pleased to contribute to an effort to showcase New York's research community. The initiative included the landmark publication of "New York: A Science State of Mind" and a November 18th gala in Manhattan.

CSHL teamed with Research!America, the Society of Neuroscience, Elsevier, and George Washington University to organize the "Research Matters Communications Workshop" for early-career scientists in Washington, D.C. on October 9. The event included a plenary session led by the Alan Alda Center for Communicating Science at Stony Brook University, a panel discussion that included journalists from CNN, Reuters, and NPR, and a session with current and former congressional staff. CSHL Assistant Professors Mikala Egeblad, Alex Krasnitz, and Steve Shea participated as part of the Public Affairs Department's program to help researchers enhance communication with nonscientific audiences.

Jim and Liz Watson

Looking Forward

2013 marked the anniversaries of two game-changing events for this institution and the world. Sixty years ago, James D. Watson and Francis Crick co-discovered the double helix structure of DNA and set off a revolution in biology and medicine. Forty-five years ago, Jim and his wife Liz came to Cold Spring Harbor Laboratory, a time when total staff numbered only 100 and the total operating budget was barely $3 million. With their foresight and leadership what in 1968 was a fledgling research institution became the modern day powerhouse in molecular biology and genetics that is today's Cold Spring Harbor Laboratory.

Dr. James Watson

"Now we are completely different," wrote Jim, looking back on what he had accomplished since taking on the challenge of leading CSHL in 1968. "The science we do, the demanding excellence of our courses and meetings, and the high quality of our publishing program convey to the world outside the aura of a quality postgraduate university. We worry not about becoming good, but instead on how to ensure that we continue to carry out science at the highest possible level" (1977 CSHL Annual Report). We thank Jim and Liz for their continuing dedication to the Laboratory and its continued success. The CSHL family of faculty, students, and employees feted Jim and Liz at a special 1960s-themed picnic on Blackford lawn in summer 2013.

To mark the anniversary of Jim and Francis Crick's DNA discovery and the fact that Jim was invited to CSHL to present the paper for the first time in public at the 1953 Qualitative Biology symposium on viruses, the Meetings & Courses Program organized a special 4-day meeting that began on the discovery's anniversary day of February 28. This meeting was called "From Base Pair to Body Plan" and was organized by Dean Alex Gann, Professor Rob Martienssen, and Meetings & Courses Program Executive Director David Stewart, with guest speakers including Nobel Prize winners Christiane Nüsslein-Volhard, Elizabeth Blackburn, Carol Greider, Craig Mello, and Sir John Gurdon. Celebration of the anniversary included the redecoration of the CSHL bar to look like the famous Cambridge pub, the Eagle, and a gala at Oheka Castle.

Jim and Liz inspired a culture that opened the doors of this institution to participation from the local community and beyond—bringing the intellectual expertise and financial generosity of private philanthropists who have been invaluable to the evolution of CSHL.

The last 60 years at Cold Spring Harbor have yielded great advances in biology. CSHL is now poised for even bigger breakthroughs that will undoubtedly change the world for the better.

Bruce Stillman, Ph.D., F.R.S.
President and Chief Executive Officer

CHIEF OPERATING OFFICER'S REPORT

W. Dillaway Ayres, Jr.

As Cold Spring Harbor Laboratory's lead administrator, my overriding objective for the past 15 years has been consistent: to create the structure that a rapidly growing enterprise requires to operate efficiently and deal with increasing regulation, without compromising a uniquely productive academic culture. Although not listed on the balance sheet, the Laboratory's culture is its most valuable asset.

In 1968, Jim Watson began establishing a culture at Cold Spring Harbor Laboratory carefully designed to foster collaboration, intensity, entrepreneurism, risk-taking, productivity, and, above all, excellence. In other words, an environment ideally suited to doing great science. This culture endures. It has been carried forward by Bruce Stillman and is sustained by many "culture carriers" here, be they facilities personnel, educators, or elite research investigators. We understand what makes the institution special and we work hard to preserve it. This work is not without challenges that sometimes shift unpredictably. Initially, my objective was to preserve culture in an environment characterized by extraordinary growth—the doubling of the National Institutes of Health (NIH) budget and substantial expansion of infrastructure and scientific staff here at the Laboratory. Now we face the opposite challenge—adjusting to a dramatic decline in federal funding that threatens America's superiority in basic science and biomedical research.

Administratively, we must make difficult decisions in response. Aggressive expense management has become a constant. Annual cost-of-living increases are no longer a given. For the first time, the decision was made to cut back on nonresearch positions at the Laboratory. This causes human hardship and puts more pressure on our dedicated staff. Culture preservation becomes an issue of maintaining morale and optimism in an increasingly difficult environment—a very different kind of managerial challenge.

Fortunately, we are benefiting from healthy growth in our endowment funds. This results from three factors: strong appreciation in public equities since the financial crisis in 2008, a revised investment strategy implemented by the Laboratory's Investment Committee during the last several years, and generous philanthropic donations to endowment. Return on investment for the 2013 calendar year was +16.5%, placing us in the upper performance tier of university endowments. Year-end market value was $385 million, another all-time high for the Laboratory. In addition, we are fortunate to have booked another $80 million in endowment pledges receivable to date through fund-raising. The importance to the future of the institution of increasing endowment cannot be overstated and remains atop our list of priorities.

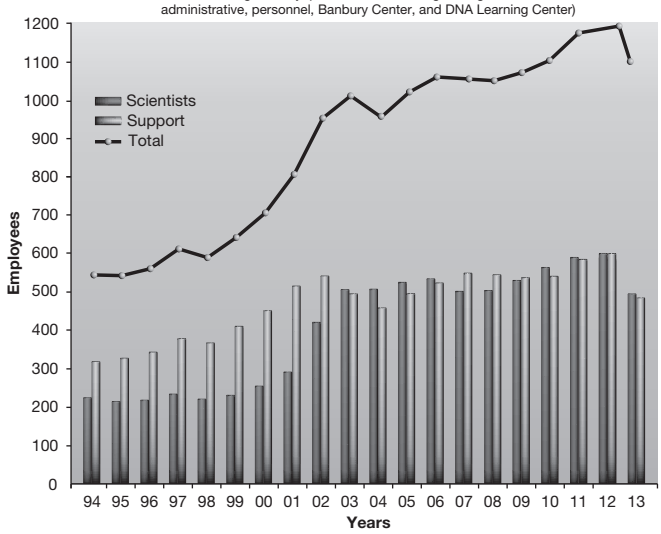
Our research and education programs remain strong. We continue to attract the very best faculty, postdocs, and students. These strengths manifest in higher than average success rates with federal grant awards. This, combined with substantial private support and a growing endowment, allows the academic programs to move forward unencumbered. We are more excited than ever about the quality and progress of our work and its promise for human health.

Our community is a cohesive one. We work hard to maintain the culture that has and continues to make Cold Spring Harbor Laboratory so productive, and we do so now in the face of some daunting challenges. We are fond of saying that "science never sleeps."

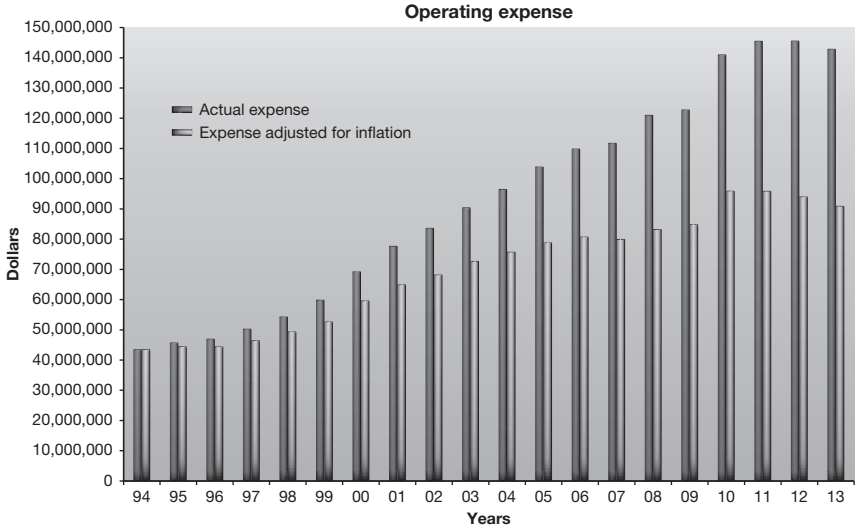
W. Dillaway Ayres, Jr.
Chief Operating Officer

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)

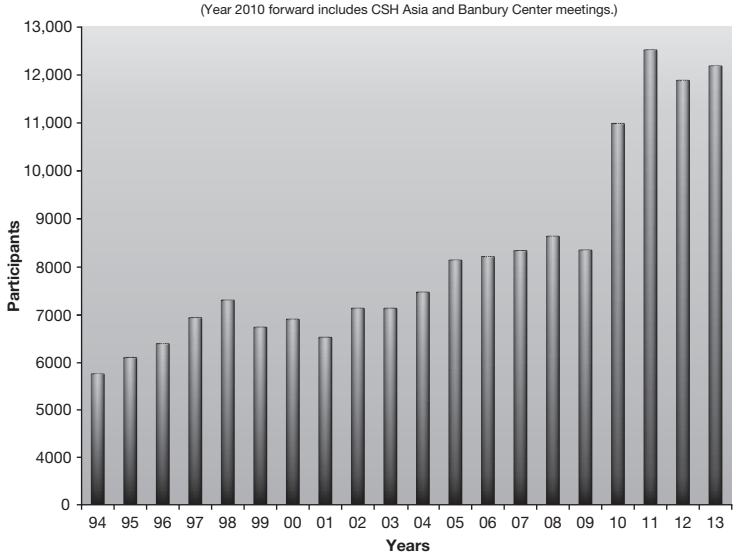


Operating expense



Meetings & Courses Participants

(Year 2010 forward includes CSH Asia and Banbury Center meetings.)



Long-Term Service

Front row (left to right) Maryliz Dickerson, Margaret Falkowski, Ronnie Packer. *Second row (left to right)* Patricia Maroney, Grigori Enikolopov, Christopher McEvoy, Margot Bennett. *Third row (left to right)* Michael Wigler, Francis Bowdren, Arne Stenlund, James Watson. *Fourth row (left to right)* Salvadore Henriquez, Bruce Stillman, David Spector, Daniel Jusino.

The following employees celebrated milestone anniversaries in 2013:

45 years	James Watson
35 years	Patricia Maroney, Christopher McEvoy, Michael Wigler
30 years	Margaret Falkowski, Daniel Jusino
25 years	Maryliz Dickerson, Grigori Enikolopov, Ronnie Packer, Francis Bowdren, Margot Bennett, Salvador Henriquez, Margaret Stellabotte, Jeffrey Goldblum, Arne Stenlund
20 years	David Stewart, Wayne Pav, Deborah Aufiero, Patricia Brady
15 years	Paul Edwards, Jesus Magana, Louis Hunter, Amanda McBrien, Michael Riggs, Drew Comer, Jeffrey Klaverweiden, Sabrina Boettcher, Thomas McIlvaine, W. Dillaway Ayres, Marja Timmermans, Ming Wang, Lari Russo, Susana Roman, Lifang Zhang

RESEARCH

CANCER: GENE REGULATION AND CELL PROLIFERATION

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 the alterations in these pathways that give rise to diseases such as cancer (as in the alterations in mitogenic pathways in melanoma). Hammell and colleagues approach this elemental problem by 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 use the small nematode *Caenorhabditis elegans*, applying forward and reverse genetic approaches. 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. They are performing high-throughput screens to identify cellular factors that allow these re-wiring events to occur, with the idea that these components would make ideal therapeutic targets to complement existing clinical strategies.

Many of the best cancer drugs are highly toxic chemotherapeutics that kill normal and malignant cells alike. In contrast, targeted compounds that recognize cancer-specific pathways represent a kind of silver bullet that would be able to distinguish tumor cells from their healthy counterparts. A few targeted drugs have been identified, such as the BRAF inhibitor vemurafenib for melanoma, and initially these agents were highly promising. But patients rapidly relapsed as their cancers became resistant to treatment.

Molly Hammell is working to tackle this problem, known as "acquired resistance," in melanoma. Her lab, in collaboration with the Wistar Institute, combines the power of systems-level, high-throughput data analysis with patient-derived tumor samples. Hammell has developed computational algorithms for the integration of multiple types of high-throughput sequencing data into gene regulatory circuits. She is now applying these methods to explore the global changes in gene regulation that enable melanoma cells to bypass inhibitors of the BRAF signaling pathway, including DNA mutations and epigenetic modifications. Her work will identify the most clinically relevant pathways of interest for additional therapeutic approaches to inhibit tumor growth in melanoma. In addition to her work on melanoma, Hammell is using her expertise in bioinformatics in collaboration with other members of the CSHL community (including Marja Timmermans, Josh Dubnau, and Greg Hannon) to understand gene regulation in diverse systems, from maize to *Drosophila*.

In **Leemor Joshua-Tor**'s lab, researchers study the molecular basis of nucleic acid regulatory processes using the tools of structural biology and biochemistry. One such regulatory process is RNA interference (RNAi), in which a small double-stranded RNA triggers gene silencing. Joshua-Tor and her team offered critical insight when they solved the crystal structure of the Argonaute protein and identified it as the long-sought Slicer. They then went on to explore the mechanism of the slicing event. The structure of human Argonaute 2 (hAgo2) bound to a microRNA (miRNA) guide allowed Joshua-Tor and her colleagues to understand how mRNA is cleaved during RNAi. This year, members of the Joshua-Tor lab explored the function of a very similar protein, called Argonaute 1, that has no slicing ability, even though it is almost identical in structure to the slicing

hAgo2. Using biochemical methods and mutational analysis, they were able to identify key parts of the protein that are required for slicing activity. The lab also studies the generation of PIWI-interacting RNAs (piRNAs), which serve to protect the genome of germ cells. With colleagues in the Hannon lab, Joshua-Tor's team also determined the structure and function of Zucchini, a key nuclease in the initial generation of piRNAs in fruit flies. In other work, the lab is exploring the mechanisms of heterochromatin formation and gene silencing through the study of a protein complex called RNA-induced initiation of transcriptional gene silencing (RITS). 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.

Adrian Krainer's lab studies the mechanisms of RNA splicing, ways in which they go awry in disease, and the means by which faulty splicing can be corrected. In particular, they study splicing in spinal muscular atrophy (SMA), a neuromuscular disease that is the leading genetic cause of death in infants. In SMA, a gene called *SMN2* is spliced incorrectly, making it only partially functional. The Krainer lab is able to correct this defect using a potentially powerful therapeutic approach. It is possible to stimulate protein production by altering mRNA splicing through the introduction of chemically modified pieces of RNA called antisense oligonucleotides (ASOs) into the spinal cords of mice. Previously, using ASOs in mice carrying a transgene of human SMN2, they developed a model for SMA using a technique they called TSUNAMI (shorthand for targeting splicing using negative ASOs to model illness). This year, they used the method to develop a mouse model for adult onset SMA, and they are currently working to develop models for the study of other diseases caused by splicing defects, including familial dysautonomia. The Krainer lab has also worked to shed light on the role of splicing proteins in cancer. They have found that the splicing factor SRSF1 functions as an oncogene stimulating the proliferation of immortal cells. This year, they were surprised to find that SRSF1 can actually stop cell growth by stabilizing a powerful tumor suppressor protein, called p53—suggesting that the cell is responding to the aberrant SRSF1 activity. This discovery offers insight into how tumors arise and the pathways that lead to transformation.

David L. Spector's laboratory studies the spatial organization and regulation of gene expression. Their recent studies demonstrated an increase in random monoallelic gene expression upon the differentiation of mouse embryonic stem cells (mESCs) to neural progenitor cells (NPCs). These data support a model where stochastic gene regulation during differentiation results in monoallelic gene expression, and for some genes, the cell is able to compensate transcriptionally to maintain the required transcriptional output of these genes. Therefore, random monoallelic gene expression exemplifies the stochastic and plastic nature of gene expression in single cells. In addition, the Spector lab is characterizing long nuclear retained noncoding RNAs (lncRNAs) that exhibit altered levels of expression as mESCs transition from the pluripotent state to NPCs, and they are studying lncRNAs that are misregulated in cancer. Their efforts have focused on *Malat1* lncRNA, which is one of the most abundant noncoding RNAs. The Spector lab previously identified a novel mechanism of 3′-end processing of this RNA. Current studies have revealed that altered levels of *Malat1* lncRNA impact breast cancer initiation and progression. Studies are currently under way to elucidate the mechanism of action of this abundant nuclear retained lncRNA.

Arne Stenlund and colleagues have obtained a detailed understanding of processes required for initiation of DNA replication from the papillomavirus, using this system to gain a general biochemical understanding applicable in other systems. 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 they divide in two. Working with yeast and human cells, Stillman and colleagues have identified many of the cellular proteins that function at the DNA replication fork during the S phase, the portion of the cell-division cycle when DNA synthesis occurs. Among these proteins are those that facilitate the assembly of chromatin, the protein–DNA complexes that form the chromosomes. The prime focus of current research, however, is the mechanism that initiates the entire process of DNA replication in eukaryotic cells. At the heart of this mechanism is a protein that binds to "start" sites on the chromosomes, called the origin recognition complex, ORC. This year, the Stillman lab was part of a collaboration that determined the cryo-EM structure of ORC proteins in complex with a group of proteins, called a helicase, that unwind DNA during replication. This image offers molecular insight into how the helicase is loaded onto DNA. Stillman's research also focuses on the process by which duplicated chromosomes are segregated during mitosis. The team has found ORC at centrosomes and centromeres, structures that orchestrate chromosome separation during mitosis. Stillman's team has discovered that mutations in the largest protein found in this complex, Orc1, alter the ability of ORC to regulate both DNA replication and centrosome duplication. These mutations have been linked to Meier–Gorlin syndrome, a condition that results in extreme dwarfism, small brain size, and related characteristics of abnormal growth.

Acute myeloid leukemia (AML) is a particularly devastating and aggressive blood cancer that is currently incurable in 70% of patients. Research in **Chris Vakoc**'s lab seeks to understand this disease as well as others, such as lymphoid leukemias and epithelial tumors, by studying them at the level of genomic regulation. He is particularly interested in the proteins that regulate chromatin in the nucleus of the cell. To identify proteins involved in AML, which may also be targets for drug therapy, he deploys large-scale genetic screens using RNA interference (RNAi) as well as genetically engineered mouse models that display the hallmarks of human cancer. In collaboration with Jay Bradner at the Dana-Farber Cancer Institute, Vakoc has shown that the small-molecule drug candidate JQ1 has potent anti-AML activity. It works by suppressing the protein Brd4, which is a critical regulator of the potent oncogene c-*Myc*. JQ1 is currently in clinical trials as a therapeutic strategy for AML. Vakoc's team identified other chromatin regulators, including PRC2 and RNF20, that if blocked with small-molecule inhibitors may halt AML. This year, Vakoc's lab gained new insights into the mechanism behind these drug targets when he found that Brd4 and other proteins required for AML bind to enhancers, short stretches of DNA more than a million bases away from the oncogene c-*Myc.* When Brd4 binds to the enhancer, it bends the DNA within the nucleus so that it touches the c-*Myc* region of the genome and activates it, causing cells to proliferate without restraint. Understanding how these proteins function in AML will allow researchers to design more effective and safer therapies to treat this intractable cancer.

THE COUPLING OF TEMPORAL GENE EXPRESSION AND DEVELOPMENTAL CELL-FATE SPECIFICATION

C.M. Hammell C. Aguirre-Chen K. Doerfel
C. Carlston R. Perales

The overarching goal of our laboratory is to understand how the precise timing of gene expression is coordinated throughout development. We are approaching this problem by using forward genetics and the model organism *Caenorhabditis elegans*. One of the main advantages of using this model is that these tiny worms have an invariant cell lineage that, in wild-type animals, results in the exact production of 959 somatic cells. Even more surprising is the fact that each of the cell divisions that give rise to the various tissues and organs of an adult animal occurs with incredible precision and at defined times. This suggests that the timing of development events is also hardwired and that the proper timing of gene expression contributes to an organism's fitness. We are exploiting the experimental tractability of this system to identify the genes that orchestrate the temporal aspects of development.

After embryogenesis, *C. elegans* larvae hatch from their eggs, begin foraging for food, grow in size, and develop in a stereotyped fashion. This period of development, called the juvenile or larval period, is characterized by dramatic organismal growth and the production of a variety of new organs that are used in adulthood. Larval development is divided into four stages that are punctuated by molts. Molting is an essential process, and its importance in coordinating the additional cellular aspects of development is underscored by the numerous cell divisions that are coupled to this process. Importantly, distinct patterns of cell division are executed at specific stages, and these temporal patterns of cell-fate specification are essential for normal morphogenesis and behavior.

Pioneering work from the Ambros and Ruvkun laboratories identified a conserved set of genes, called heterochronic genes, that are required for establishing the normal sequence of stage-specific cell-fate decisions. Mutations in heterochronic genes result in temporal cell-fate transformations that include either an inappropriate skipping or reiteration of stage-specific patterns of cell divisions. Many heterochronic genes encode transcription factors and RNA-binding proteins that turn on or turn off gene expression patterns in a stage-specific manner. Several protein-coding genes, such as *lin-14*, *lin-28*, *hbl-1*, and *lin-41*, are important for controlling temporal patterning and are regulated by microRNAs (miRNAs). In this context, miRNAs are expressed and operate at defined times during postembryonic development and function as molecular switches to curtail earlier patterns of development and promote the emergence of later gene expression profiles (Fig. 1). Throughout postembryonic development, the expression of individual heterochronic miRNAs is regulated at both the transcriptional and posttranscriptional levels. Although the detailed study of the regulatory requirements of individual miRNAs has produced a wealth of information, it is not known whether a higher level of organization coordinates the precise temporal expression of all heterochronic genes.

The stringent requirement of normal temporal patterning for the precise control of miRNA activity

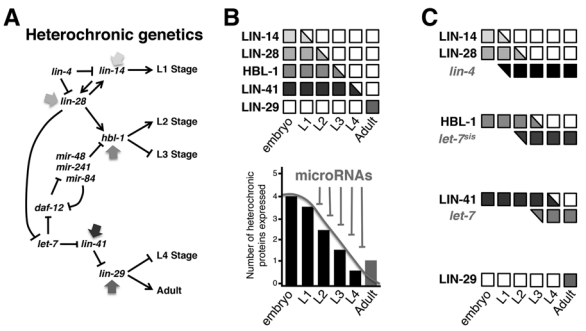


Figure 1. (*A*) The genes regulating the heterochronic pathway that control specific cell-fate transitions are ordered into a large, linear gene-regulatory logic. (*B*) The ordered down-regulation of most heterochronic genes is orchestrated by the sequential activity of miRNAs. (*C*) The transcriptional regulation of miRNAs and the subsequent down-regulation of their targets is accomplished by three separate families of miRNAs.

provides a unique genetic context to identify genes that regulate this process. We determined that there might be a set of genes that normally function to dampen miRNA expression in order to maintain miRNA levels within strict physiological ranges. These genes would be important for maintaining the normal robustness of the *C. elegans* developmental lineage. This is because mutations in miRNAs that control temporal cell fate are dosage dependent: Overexpression or underaccumulation of heterochronic miRNAs during development results in reciprocal transformations of cell-fate specification. Mutations that alter the function of these genes could be identified in forward genetic screens because mutant animals would display precocious heterochronic phenotypes due to the overexpression of one or more miRNAs. Alternatively, removal of these components in genetic backgrounds that underaccumulate miRNAs would alleviate phenotypes associated with reduced miRNA target regulation.

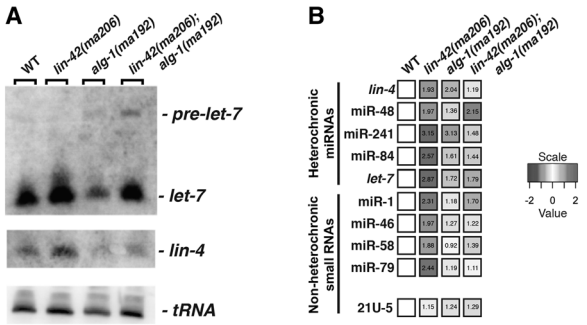


Figure 2. *lin-42* mutations lead to the overexpression of several miRNAs. (*A*) Small RNA northern analysis of 20 µg of total RNA extracted from morphologically staged, young adult, wild-type *lin-42(ma206), alg-1(ma192),* and *lin-42(ma206);alg-1(ma192)* animals. Blots were probed sequentially for the indicated miRNAs. tRNAGly serves as a loading control. (*B*) The results of miR-TaqMan assays to quantify the levels of mature miRNAs in wild-type, *lin-42(ma206), alg-1(ma192),* and *lin-42(ma206);alg-1(ma192)* animals. Notice that *lin-42(ma206)* displays the highest levels of miRNAs relative to the other genotype backgrounds. Data represent three biological replicates with three technical replicates each. Heat map colors are shown as log2 scale as indicated and within each individual assay. Red indicates an increase in miRNA expression, and blue indicates a reduction in mature miRNA levels. Numbers within each box indicate standard fold change when compared to wild-type samples.

lin-42 Negatively Regulates the Biogenesis of Postembryonically Expressed miRNAs

In a set of pilot screens geared to identify suppressors of hypomorphic miRNA mutants, we identified five alleles of the *lin-42* gene. When outcrossed, animals harboring *lin-42* mutations display profound heterochronic phenotypes including the precocious expression of adult characteristics one stage earlier than normal. These phenotypes allowed us to clone the mutations in the *lin-42* gene that cause these phenotypes. The *lin-42* gene is complex in that it produces many alternatively spliced transcripts, and these mRNAs are derived from two different promoters. Furthermore, both *lin-42* promoters are activated in an oscillatory fashion once each stage during postembryonic development. At the protein level, LIN-42 encodes the *C. elegans* homolog of the human and *Drosophila PERIOD* gene involved in circadian gene regulation. As with PERIOD, LIN-42 protein oscillates in expression and is localized to the nuclear compartment. Analysis of miRNA expression levels from *lin-42* mutant animals suggests that LIN-42 broadly alters miRNA biogenesis. Surprisingly, mutations in the *lin-42* gene lead to the overexpression of most postembryonically expressed miRNAs while having little or no effect on the expression of other small regulatory RNAs (Fig. 2). In fact, we found that the precocious phenotypes of *lin-42* mutants result from the inappropriate overexpression of heterochronic miRNAs.

To determine how *lin-42* regulates miRNA expression, we developed a variety of transcriptional reporters for various miRNAs. These reporters are unique in that each miRNA promoter drives the expression of a constitutively destabilized green fluorescent protein (GFP). These unique tools enabled us to determine that the promoters of many miRNAs are transcriptionally activated in a pulsatile manner and that these patterns are very similar to the expression of *lin-42*. We then sought to characterize the expression of various miRNAs in *lin-42* mutants. We focused on characterizing miRNA expression in *lin-42* mutants that delete the highly conserved PAS domain that distinguishes LIN-42 as the *C. elegans* homolog of human and *Drosophila* PERIOD. Mutation or deletion of this domain in the PERIOD protein completely abolishes periodic circadian gene expression. Surprisingly, the oscillatory patterns of miRNA expression are still maintained in *lin-42*

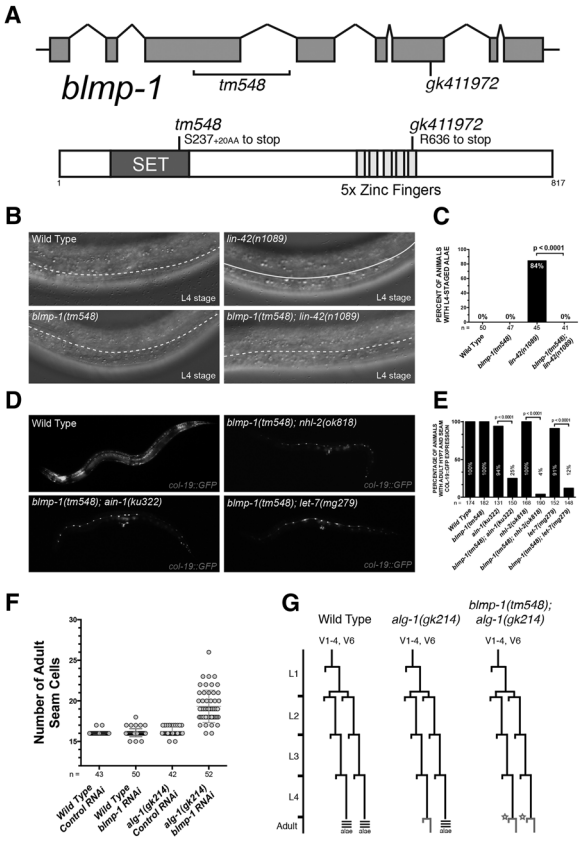


Figure 3. *blmp-1* is a new heterochronic gene. (*A*) *blmp-1* encodes an 817AA zinc-finger transcription factor. The *tm548* allele of *blmp-1* deletes a central portion of the *blmp-1* gene and causes a premature stop in the BLMP-1 protein at amino acid 237. Therefore, *tm548* is a presumptive null allele of this gene. (*B,C*) *blmp-1* mutations strongly suppress the precocious alae phenotypes of *lin-42*. Images show the lateral hypodermis of indicated animals and the L4 stage. The cuticles of *lin-42(n1089)* animals have adult-specific alae. Removing *blmp-1* in the *lin-42(n1089)* background completely suppresses these phenotypes. (*D,E*) *blmp-1(tm549)* mutations dramatically enhance the heterochronic phenotypes of miRISC mutants. (*F,G*) Removal of *blmp-1* function in animals that lack one of the two miRNA-specific argonautes (*alg-1*) leads to an increase in the number of lateral hypodermal stem cells.

mutant animals. Although the temporal patterns of miRNA expression are maintained in *lin-42* mutants, they were dramatically overexpressed. These findings explain why *lin-42* mutants display pleiotropic heterochronic phenotypes and further suggest that the PAS domain of LIN-42 functions to negatively regulate transcription.

Identification of *blmp-1*, a New Heterochronic Gene That Promotes the Expression of Heterochronic miRNAs

The precocious developmental phenotypes of *lin-42* mutants are due to elevated transcription of multiple miRNA genes and the early down-regulation of specific miRNA targets. We reasoned that *lin-42* likely antagonizes the function of one or more transcription factors that promote the temporal expression of heterochronic miRNAs and that reducing the activity of this transcription factor could alleviate the precocious phenotypes of *lin-42*. To identify these candidate transcription factors, we systematically depleted them via RNA interference (RNAi) and determined whether the reduction of their expression could reduce the penetrance of *lin-42* precocious phenotypes. This strategy identified the conserved zinc-finger/SET-domain-containing transcription factor BLMP-1. Analysis of these genetic interactions with a deletion allele confirmed these results. Furthermore, *blmp-1* mutants display retarded heterochronic phenotypes and strongly enhance the developmental phenotypes of mutations that alter miRNA-induced silencing complex (miRISC) components (Fig. 3). Future experiments are aimed to determine the direct nature of *blmp-1*-mediated gene regulation and how *blmp-1* expression is coordinated with other heterochronic genes.

INTEGRATING GENOMIC DATA SETS INTO GENE REGULATORY NETWORKS

M. Hammell Y. Hao Y. Jin A. Patel O. Tam
Y.-J. Ho E. Paniagua J. Regalado Perez

Cellular function is dictated by the complex interplay of gene regulatory networks that control gene expression. These networks are incredibly robust to small or temporary fluctuations in the environment, yet they retain the ability to change and rewire themselves in response to large-scale or long-term changes in external stimuli. This plasticity in genetic networks is mediated by multiple types of regulatory factors, such as transcription factors and noncoding RNAs, which respond coordinately to changes in cellular signaling. Thus, gaining a systems-level view of how these factors combine to produce a specific cellular outcome requires distilling multiple types of genomic profiling data into an integrated model of genetic signaling pathways. Our lab uses computational algorithms to integrate multiple types of genomic and transcriptomic profiling data into models of regulatory rewiring events in human disease. This includes an emphasis on developing novel tools for the statistical analysis of high-throughput data, developing novel algorithms for modeling the flow of signals through genetic pathways, and, importantly, testing these models using the tools of molecular genetics.

The ultimate goal is to understand how human diseases such as cancer take advantage of the cell's innate propensity for plasticity to rewire these regulatory networks into programs that serve the needs of the cancer cells.

In particular, members of our lab use a combination of computational and experimental methods to better understand how gene expression is regulated in plant and animal genomes. Much of this work involves tight collaborations with other experimental groups at CSHL. One example involves a collaboration with Marja Timmermans' lab to understand the diversity of small RNA regulators in the maize genome and how these small RNAs dictate organ patterning in development. Another collaboration with Josh Dubnau's lab is exploring the contribution of transposons to human neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). A collaboration with Greg Hannon's lab probes the molecular mechanisms of transposon control in animals. An independent project in our lab combines both computational and experimental techniques to understand how melanoma cells develop resistance to targeted inhibitors of the melanoma cell growth pathways. Although the experimental systems for each of these projects vary greatly, each project seeks to understand how gene regulation contributes to maintaining cellular function.

Mechanisms of Acquired Drug Resistance in Melanoma

A. Patel, J. Regalado Perez

The genetic basis of melanoma development is fairly well understood, with activating mutations in the oncogene *BRAF* occurring in a majority of melanoma patients (Hodis et al., [2012]). Specific inhibitors that target activated *BRAF* as well as the downstream MAP/ERK (mitogen-activated protein kinase/extracellular signal-related kinase) signaling pathway have been developed, which dramatically reduce the growth of melanoma cells in patients. However, the effects of these drugs typically extend patient life span for 6 mo or less, as the tumors rapidly develop resistance to these targeted therapies (Villanueva et al., [2011]). Although some tumors resistant to BRAF inhibitors acquire additional genetic lesions that elevate ERK or AKT signaling (Alcala et al., [2012]), an astounding number of therapy-resistant cell lines establish resistance without significant alteration of the genome (M. Herlyn, pers. comm.). Furthermore, serial passage of some of these cell lines in drug-free media results in the re-acquisition of sensitivity to the targeted therapeutics, suggesting that the resistance mechanism in these cases is likely to be due to changes in gene regulation rather than secondary acquired

mutations. There are many factors that could be mediating this reversible acquired resistance phenotype, the most likely of which would involve alterations in the expression of small RNAs, alternative splicing of key transcripts in the MEK/ERK or AKT signaling pathways, or chromatin-modifying enzymes that alter DNA methylation or histone modification. Members of our lab are using genomic and transcriptomic profiling studies to identify the gene regulatory factors that accompany acquired resistance to BRAF inhibitors. These experimental data sets are fed into custom algorithms designed to integrate these disparate data types into a systems-level view of the cellular signaling pathways that underlie melanoma growth and BRAF inhibitor resistance.

Small RNA Pathways in Maize

O. Tam [in collaboration with M. Timmermans, Cold Spring Harbor Laboratory]

The maize genome has remained relatively unexplored despite the importance of maize as one of the early genetic model organisms. Preliminary characterizations of the maize genome and transcriptome suggest that substantial differences exist, both between maize ecotypes as well as the well-characterized plant model *Arabidopsis* (Springer et al., [2009]; Eveland et al., [2010]). Furthermore, these maize inbreds exhibit dramatic phenotypic differences in response to mutations in small RNA biogenesis factors, suggesting extensive evolution in both small RNA function and activity. In collaboration with the Timmermans lab, my group has undertaken a project to characterize the dynamics of small RNA expression and activity in a variety of maize tissues and inbreds. The focus of this project is to determine the small RNA-mediated regulatory circuits that give rise to inbred-specific phenotypic diversity both within the maize species and within the larger group of grasses. Our analysis of maize *trans*-acting small interfering RNAs (tasiRNAs), a group of mobile endogenous 21-nucleotide siRNAs, has revealed that in maize shoots, tasiRNAs are generated through just a single genetic pathway and function solely in leaf development. In contrast, the many tasiRNAs found in *Arabidopsis* are processed via several distinct biogenesis pathways and function in development as well as pathogen defense. This same analysis also identified a new class of 22-nucleotide phased siRNAs, produced by an unidentified pathway that does not overlap with the tasiRNA biogenesis pathway. This class of small RNAs is entirely novel, with no homologs in other sequenced plants and no defined mechanism for biogenesis or activity. Finally, only 25% of the known microRNAs (miRNAs) from *Arabidopsis* are conserved in maize, leaving a wealth of novel miRNAs to explore. Members of our lab are collaborating to provide computational analysis of the profiles of small RNAs and their targets across maize ecotypes. Our lab has developed novel algorithms for the identification of phased small RNAs, for miRNA gene identification, and for miRNA target interaction analysis. These algorithms are being applied to deep sequencing data sets of small RNAs and mRNAs isolated from developing maize tissues.

Transposon Control Systems in Animals

Y. Jin, O. Tam [in collaboration with G. Hannon and J. Dubnau, Cold Spring Harbor Laboratory]

TDP-43 is an RNA-binding protein that is known to control proper splicing and translation of many RNA targets in neurons. Mutation of TDP-43 has been associated with a variety of neurodegenerative diseases including ALS, frontotemporal lobar degeneration (FTLD), and Alzheimer's disease (AD) (Cohen et al., [2011]). However, the normal function of TDP-43 in neuronal development and maintenance has not been fully characterized, and few of its mRNA targets have been definitively associated with the neurodegenerative diseases that result from loss of TDP-43 function (Da Cruz et al., [2011]). In collaboration with the Dubnau lab, my group has explored the novel hypothesis that TDP-43 normally has a large and hitherto uncharacterized role in regulating the expression of transposable elements (TEs), mobile genetic elements whose unregulated expression leads to genetic instability as well as cellular toxicity. Members of the Dubnau lab have shown that TE transcripts are elevated upon expression of mutant, aggregate-prone forms of human TDP-43 in the fly brain and that neurodegeneration results from expression of this human TDP-43 protein. Members of my group have shown that TDP-43 binds widely to TE transcripts in mammals and that TDP-43 binding to TEs is lost in human patients diagnosed with FTLD (Li et al., [2012]), a disease characterized by TDP-43 proteinopathy.

Although these studies support a role for TDP-43 in regulating TE expression, our future goals are centered on defining a causal role for TDP-43-mediated regulation of TEs in neurodegenerative disease. One important element of this project will be the identification of how TDP-43 interacts with the small RNA regulators of TE expression known to be involved in controlling TE mobility. The Dubnau lab is currently exploring the molecular mechanism behind TDP-43 function in the fly and mouse model organisms, where many genetic mutants are available to carefully describe the factors that mediate TDP-43 involvement in TE regulation, biogenesis, and activity. My group is pursuing a parallel approach in close collaboration with the Dubnau lab to quantify the extent to which TDP-43 contributes to TE regulation as compared to its other mRNA targets and the potential contribution of each function to clinical outcomes.

In a separate collaboration, we work closely with members of the Hannon lab to better understand the biological mechanisms of transposon control in animals. This basic research into the factors that regulate transposon activity sets the foundation for understanding disease processes affected by transposon misregulation. Most families of TEs are highly distinct at the primary sequence level and use different strategies for their propagation, challenging the ability of host genomes to discriminate transposons from endogenous genes (Levin and Moran, [2011]). Several recent studies have indicated that a small RNA-based innate immune system, the Piwi-interacting RNA (piRNA) pathway, addresses the challenge of transposon recognition and silencing. In germ cells, primary piRNAs are loaded into Piwi and Aubergine to control transposons through a posttranscriptional mechanism. However, several factors, including Piwi's nuclear localization, have suggested that Piwi might also regulate its targets by controlling their transcription. We therefore probed the contributions of Piwi to transposon silencing in germ cells and in their somatic support cells using tissue-specific piwi knockdowns.

Integrated analyses of steady-state RNA levels by RNA sequencing (RNA-Seq), and measurements of nascent RNA synthesis using global run-on sequencing (GRO-Seq), chromatin marks by chromatin immunoprecipitation combined with deep sequencing (ChIP-Seq), and small RNA levels indicated multiple roles for Piwi in the piRNA pathway in both the germline and the soma. In the soma, our data support a model where Piwi silences the transcription of targeted elements, such that transcriptional gene silencing (TGS) is the dominant form of Piwi activity. In germ cells, our data revealed that Piwi both acts via TGS and exerts unexpected impacts on piRNAs that occupy Aubergine and Ago3, indirectly reducing the capacity of the entire piRNA pathway to silence transposons via posttranscriptional gene silencing (PTGS). These data sets add new complexity to our understanding of Piwi function, which has primarily been studied for its direct role in PTGS. Ongoing experiments will seek to understand the mechanisms by which Piwi contributes to TGS and how Piwi indirectly affects other moderators of PTGS.

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STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

L. Joshua-Tor E. Elkayam A. Epstein C. Faehnle S. Goldsmith J. Ipsaro M. Jacobs C.-D. Kuhn C. Kuscu S.-J. Lee M. Morales A. Tocilj D. Wah J. Walleshauser M. Wang

We study the molecular basis of nucleic acid regulatory processes by using the tools of structural biology, biochemistry, and biophysics 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, biophysics, and molecular biology allow us to study properties that can be correlated to their function and biology.

Mechanisms of RNAi

C. Faehnle, E. Elkayam, J. Ipsaro, C. Kuhn, C. Kuscu, J. Walleshauser [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 to get a true mechanistic understanding of these pathways, we must understand how the components of the RNAi machinery work at a molecular level. Therefore, we embarked on structural, biochemical, and biophysical studies of key proteins in the RNAi pathway.

The Making of a Slicer: Structure and Activation of Human Argonaute-1

In humans, Argonaute-2 (hAgo2) is the key effector protein in the RNA-induced silencing complex (RISC) that was identified as Slicer, the endonuclease that cleaves a target RNA when the guide and target are fully complementary. Besides slicer-mediated RNAi, hAgos 1–3 are at the heart of nonslicer gene repression mechanisms involving translational repression and mRNA destabilization. In this case, partially mismatched miRNAs guide Argonaute to its target, where the recruitment of downstream silencing factors commences. Because slicing does not take place in microRNA (miRNA) silencing pathways, several mammalian Argonautes have lost their ability to slice, namely, hAgo1 and hAgo3. Most perplexing is the fact that despite an intact catalytic site in hAgo3, it still lacks slicer activity. In addition, hAgo2 can function in both a slicer and nonslicer mode. The question arises therefore as to whether a nonslicing conformation exists (for hAgo1 and hAgo3, e.g.) or is assumed (by hAgo2). To understand what makes an active slicer, we set out to characterize the structural determinants of slicing by comparing structures of human Argonaute proteins and to make an active slicer enzyme out of the otherwise inactive hAgo1. We solved the structure of hAgo1 loaded with endogenous Sf9 guide RNA to 1.75 Å and in complex with the tumor suppressor *let-7* miRNA to 2.1 Å. The structure of hAgo1 closely resembles the structures of hAgo2 in complex with guide RNAs that we and other investigators determined recently. Like hAgo2, hAgo1 is composed of the same domain organization as that of N, PAZ, Mid, and PIWI domains. The PIWI domain is similar to RNase H-like nucleases and contains the conserved DEDH catalytic tetrad required for endonuclease slicer activity. hAgo1 has a mutated DEDR active-site tetrad that, upon restoration to DEDH, remains inactive. Instead, through extensive mutagenesis, we found that mutation of a leucine to phenylalanine within a loop adjacent to the active site of hAgo1 restores slicing activity. We mutated this loop in the slicer-active hAgo2 and showed that the integrity of this region is essential for

target slicing. When we coupled this active mutation in hAgo1 with an amino domain swap with hAgo2, the target slicing activity was substantially enhanced. Conversely, the hAgo1 amino domain, when swapped into hAgo2, impaired slicing. Finally, hAgo3, which already has an intact DEDH tetrad, becomes an active slicer by swapping in the amino domain of hAgo2. These domain-swapping experiments point to a previously underappreciated function for the amino domain in Argonaute slicing activity. Intriguingly, the elements that make Argonaute an active slicer involve a sophisticated interplay between the active site and more distant regions of the enzyme. We continue to investigate the features of Argonaute that arose from our structural and biochemical work to further our understanding of Argonaute proteins in human development and disease.

RNAi and Heterochromatin Formation

RNAi pathways are also used to repress genes at the transcriptional level by guiding heterochromatin formation. Although we are just beginning to discover how metazoans use this type of mechanism for transcriptional silencing, these pathways are much better characterized in plants and fungi, in particular in *Schizosaccharomyces pombe*, where heterochromatin is essential for kinetochore assembly required for proper segregation of chromosomes during cell division. The complexes involved in heterochromatization in *S. pombe* are fairly well defined, and in most cases, only one version of each component exists. However, our understanding of this pathway has not gone much beyond the "blobology" stage either. Assembly of centromeric heterochromatin requires the RITS complex, a specialized RISC, that physically anchors small RNAs to chromatin. It consists of the Argonaute protein Ago1, the chromodomain protein Chp1, a GW-like protein Tas3, and small interfering RNAs (siRNAs) derived from centromeric repeats. We previously characterized Chp1's high-affinity binding to chromatin and showed it to be critical for establishment of centromeric heterochromatin. In addition, apart from the chromodomain, Chp1 had no recognizable domains. Our structure of the carboxy-terminal half of Chp1 with the Tas3 amino-terminal domain revealed the presence of a PIN domain in Chp1 that contributes to posttranscriptional gene silencing of subtelomeric transcripts independent of RNAi. We also realized that Chp1 and Tas3 form a tight complex, and Argonaute appears to bind more loosely. We suggested that Chp1-Tas3 provides a solid and versatile platform to recruit both RNAi-dependent and -independent gene-silencing pathways for locus-specific regulation of heterochromatin and that the reach of long and flexible regions in these two proteins might be important for contacting other nucleosomes at these loci.

The RITS complex recruits the H3K9 methylase Clr4, which is part of a large complex called CLRC. CLRC also contains the cullin Cul4 and its interacting protein Pip1, the adaptor Rik1, a WD repeat protein Dos1, and Dos2. Curiously, CLRC is an active E3 ligase in vitro, and this activity is necessary for heterochromatin assembly in vivo. Yet, the role of this E3 ligase activity is still unknown. We performed a pairwise interaction screen of the CLRC components revealing a subunit arrangement that is reminiscent of CRL4, a different cullin ring ligase complex for which a crystal structure is available. Both complexes contain the ubiquitin ligase Cul4, and sequence similarities between two other subunits were already noted. We have begun delineating structural and functional similarities. In particular, the placement of Dos1 indicated that it might have a role similar to that of DDB2 in CRL4, as a target-binding factor for the E3 ligase. We solved the structure of Dos1 and found it to be an eight-bladed β propeller with one face mediating interaction with the adaptor protein Rik1. We made a series of mutations on the opposite surface and found several regions that are required for heterochromatin silencing, underscoring the notion that Dos1 is the specificity factor for the ligase.

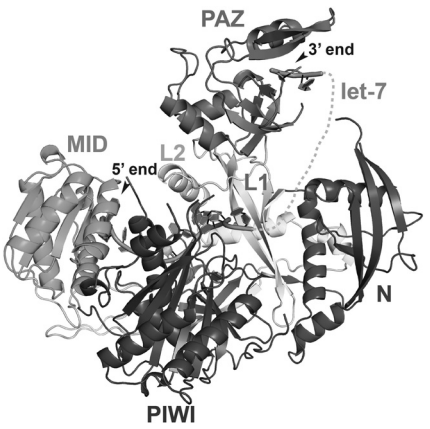

Figure 1. Structure of human Argonaute-1 in complex with the tumor suppressor miRNA let-7.

The Different Faces of E1: A Replicative Hexameric Helicase

S.-J. Lee [in collaboration with A. Stenlund, Cold Spring Harbor Laboratory; T. Ha, University of Illinois, Urbana-Champaign]

Precise replication of the genome is essential for maintaining the integrity of genomic information. As a prerequisite for 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 while moving along the DNA. Our crystal structures of the double-stranded DNA (dsDNA)-binding domain (DBD) of the replicative helicase E1 from papillomavirus in various stages of assembly led us to propose a model in which the transition from the dimer to the ultimate double hexamer results in strand separation. The loading and assembly of this protein separate the double helix, such that each hexameric helicase encircles one strand of DNA. Once assembled, the helicase uses its ATP-driven motor to translocate on the DNA or "pump" the single-stranded DNA (ssDNA) through the hexameric ring. Several competing mechanisms for helicase unwinding were proposed. Having determined a structure of hexameric E1 with ssDNA discretely bound in the central channel and nucleotides at the subunit interfaces, we showed that only one DNA strand passes through the hexamer channel and that the DNA-binding hairpins of each subunit form a spiral staircase that sequentially tracks the DNA backbone. The nucleotide configurations at the subunit interfaces indicate that each subunit sequentially progresses through ATP, ADP, and apo states, whereas its associated DNA-binding hairpin travels from the top to the bottom of the staircase, each escorting one nucleotide of ssDNA through the channel, as if six hands grab the DNA and upon ATP hydrolysis and ADP release pull it through the channel. With this unique look into the mechanism of translocation of this molecular machine along DNA, we have focused on mechanistic aspects of the enzyme in solution.

By taking a multifaceted approach including single-molecule and ensemble FRET (Förster resonance energy transfer) methods, we have found that E1 is oriented with the amino-terminal side of the helicase facing the replication fork, consistent with the crystal structure. We also showed that E1 generates strikingly heterogeneous unwinding patterns stemming from varying degrees of repetitive movements that are modulated by the DNA-binding domain. Furthermore, our studies found that DNA-binding domain promotes the assembly of E1 helicase onto a forked DNA substrate, acting as an allosteric effector of the helicase. Taken together, our studies reveal previously unrecognized dynamic facets of replicative helicase unwinding mechanisms, adding another layer of complexity in the workings and regulation of DNA replication.

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RNA SPLICING

A.R. Krainer M. Akerman, O. Anczuków, I. Aznarez, Y. Basedo, F. Bezirci, S. Brøner, W.-J. Chen, S. Das, C. Hu, X. Hua, Y. Hua, K. Ishida, M. Jensen, H.-Y. Jeon, D. Vargas Landín, K.-T. Lin, Y.-H. Liu, L. Manche, T. Nomakuchi, J. Novatt, E. Panagot, H. Rees, K. Sahashi, J. Scharner, M. Sivaramakrishnan, R.-Y. Tzeng, D. Wadler, M. Walia, J. Wu

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 protein-coding 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.

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 characteristic of all cancer cells, known as the Warburg effect. We have continued to analyze the roles of individual members of the SR protein family of splicing factors in cancer.

As part of a systematic analysis of the SRSF1–protein interaction network, we used immunoprecipitation and quantitative mass spectrometry (i-DIRT) and found a novel interaction among SRSF1, the ribosomal protein RPL5, and the ubiquitin E3 ligase MDM2. We demonstrated that SRSF1 stabilizes the tumor suppressor protein p53 by abrogating its MDM2-dependent proteasomal degradation. Moreover, SRSF1 is a necessary component of the complex, which functions in a p53-dependent ribosomal-stress checkpoint pathway. Consistent with the stabilization of p53, increased SRSF1 expression in primary human fibroblasts decreased cellular proliferation and triggered oncogene-induced senescence (OIS). These findings underscore the deleterious outcome of SRSF1 overexpression and identified a cellular defense mechanism

against its aberrant function. Furthermore, they implicated the RPL5-MDM2 complex in OIS and demonstrated a link between spliceosomal and ribosomal components, functioning independently of their canonical roles, to monitor cellular physiology and cell cycle progression.

We also collaborated with Frédéric Allain (ETH, Zurich), whose group used nuclear magnetic resonance (NMR) to solve the structure of the human SRSF1 pseudo-RNA-recognition motif (pseudo-RRMs) bound to RNA. Although pseudo-RRMs are crucial for the activity of SR proteins that have one, their mode of action was unknown. The structure revealed a very unusual and sequence-specific RNA-binding mode centered on one α-helix, instead of the β-sheet surface, which typically mediates RNA binding by RRMs. We found that the isolated pseudo-RRM was sufficient to regulate splicing of about half of the SRSF1 target genes tested, and the bound α-helix was required for this function. These results suggest that SR proteins with a pseudo-RRM frequently regulate splicing by competing with, rather than recruiting, spliceosome components, binding RNA through this unusual RRM.

We also studied another SR protein, SRSF6, and identified it as a regulator of wound healing and tissue homeostasis in skin (in collaboration with John Erby Wilkinson, University of Michigan, Ann Arbor). We found that SRSF6 is a proto-oncogene frequently overexpressed in human skin cancer, including malignant melanomas and basal cell and squamous cell carcinomas. Overexpressing doxycycline-inducible SRSF6 cDNA in transgenic mice induced epithelial hyperplasia of sensitized skin (e.g., by shaving) and promoted aberrant alternative splicing. We observed severe epidermal thickening and hair-follicle lesions, which were reversible upon withdrawal of the doxycycline inducer. Skin transplantation experiments to nontransgenic donor mice showed that the inducible SRSF6 effects are cell autonomous.

Microarray analysis identified 139 target genes with SRSF6-induced alternative-splicing changes in skin, including many genes associated with wound healing. Focusing on the *Tnc* gene coding for the extracellular matrix protein tenascin C, we demonstrated that SRSF6 binds to alternative exons in its pre-mRNA and promotes the expression of isoforms characteristic of invasive and metastatic cancer, although this particular regulatory event is independent of cell type. SRSF6 overexpression additionally resulted in depletion of $LGR6^+$ stem cells and excessive keratinocyte proliferation and response to injury. Furthermore, we showed that the effects of SRSF6 in wound healing assayed in vitro depend on the tenascin C isoforms. Thus, abnormal expression of this SR protein splicing factor can perturb tissue homeostasis in skin.

Targeted Antisense Modulation of Alternative Splicing for Therapy and Disease Modeling

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 previously developed an antisense method to increase the extent of exon 7 inclusion during splicing of *SMN2* transcripts, for therapeutic use in SMA. This translational research is being done in collaboration with Isis Pharmaceuticals. Phase II open-label clinical trials with the ASO compound, ISIS-SMN_{Rx}, are well under way; the drug, which was administered to SMA infants and children by lumbar puncture, was well tolerated at all doses tested, and dose- and time-dependent improvements in motor function were observed. Double-blind, placebo-controlled Phase III trials to establish effectiveness are being planned.

We also described the use of another antisense oligonucleotide (ASO) complementary to exon 7 that exacerbates *SMN2* missplicing to phenocopy SMA in a dose-dependent manner when administered to *SMN2*-transgenic $Smn^{-/-}$ mice. Intracerebroventricular ASO injection in neonatal mice recapitulated SMA-like progressive motor dysfunction, growth impairment, and shortened life span, with α-motor neuron loss and abnormal neuromuscular junctions. These SMA-like phenotypes were prevented by intracerebroventricular (ICV) injection of the above therapeutic ASO. We uncovered starvation-induced splicing changes—particularly in *SMN2*—that likely accelerate disease progression. These results constitute

proof of principle that ASOs designed to cause sustained splicing defects can be used to induce pathogenesis and rapidly and accurately model splicing-associated diseases. This approach, which we dubbed TSUNAMI (targeting splicing using negative ASOs to model illness), also allows the dissection of pathogenesis mechanisms, including spatial and temporal features of disease onset and progression as well as testing of candidate therapeutics. By targeting splicing of endogenous genes, the method can potentially be used to phenocopy diseases in wild-type animals.

We have now used the TSUNAMI approach to phenocopy adult-onset SMA (known as type IV SMA) in mice. We again used the above exon-7 ASO to exacerbate SMN2 missplicing and observed distinctive pathological features of adult-onset versus early-onset SMA. We demonstrated that ICV ASO injection in adult SMN2-transgenic mice phenocopies key aspects of adult-onset SMA, including delayed-onset motor dysfunction and relevant histopathological features. As in the neonate model, SMN2 missplicing increased during late-stage disease, likely accelerating disease progression. Systemic ASO injection in adult mice caused SMN2 missplicing in peripheral tissues and affected prognosis, eliciting marked liver and heart pathologies, with decreased circulating insulin-like growth factor 1 (IGF1) levels. ASO dose-response and time-course studies suggested that only moderate SMN levels are required in the adult central nervous system, and treatment with the splicing-correcting ASO showed a broad therapeutic time window.

Analyzing Splicing by Next-Generation Sequencing

A crucial step in analyzing mRNA-Seq data is to accurately and efficiently map hundreds of millions of reads to the reference genome and to exon junctions. In collaboration with Chaolin Zhang (Columbia) and Michael Zhang (University of Texas, Dallas), we developed OLego, an algorithm specifically designed for de novo mapping of spliced mRNA-Seq reads. OLego adopts a multiple-seed-and-extend scheme and does not rely on a separate external aligner. It achieves high sensitivity of junction detection by strategic searches with small seeds (~14 nucleotides for mammalian genomes). To improve accuracy and resolve ambiguous mapping at junctions, OLego uses a built-in statistical model to score exon junctions by splice-site strength and intron size. Burrows-Wheeler transform is used in multiple steps of the algorithm to efficiently map seeds, locate junctions, and identify small exons. OLego is implemented in C^{++} with fully multithreaded execution, and it allows fast processing of large-scale data. We systematically evaluated the performance of OLego in comparison with published tools, using both simulated and real data. OLego demonstrated better sensitivity, higher or comparable accuracy, and substantially improved speed. It also identified hundreds of novel micro-exons (<30 nucleotides) in the mouse transcriptome, many of which are phylogenetically conserved, and we validated them experimentally.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector G. Arun, J. Bergmann, M. Bodnar, K.-C. Chang, S. Dai, S. Diermeier, M. Eckersley-Maslin, S. Hearn, M. Hübner, R. Kumaran, J. Li, C. Zepeda-Mendoza

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 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 this multifaceted process are just beginning to be elucidated. During the past year, our research has continued to focus on elucidating various mechanisms of regulating gene expression and DNA repair and the role of long nuclear retained noncoding RNAs in development and cancer progression.

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

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

Embryonic stem cells (ESCs) undergo dramatic changes in transcription, chromatin structure, and nuclear architecture as they transition from a pluripotent state to a lineage-specific cellular program. To better understand how the unique ESC nuclear environment influences pluripotency, and vice versa, we have focused on the dynamic movements of the *Oct4* gene locus during the onset of ESC differentiation. We have found that the alleles of the *Oct4* gene locus transiently associate in the nucleus and that the timing of this allelic association event coincides with the transcriptional modulation of the *Oct4* gene.

Although differentiating ESCs can partially recapitulate embryonic development in vitro, we were interested in whether the observed *Oct4* allelic associations that we previously observed at specific time points after induction to differentiate would also occur in vivo during early mouse embryo development. In the postimplantation mouse epiblast, *Oct4* expression in the ectoderm/neuroectoderm is downregulated in an anterior to posterior manner concurrent with neural lineage specification and loss of pluripotency, such that by the 6-somite stage (~E8.5), *Oct4* expression is largely restricted to primordial germ cells. We reasoned that if *Oct4* allelic pairing was correlated with repression of the *Oct4* gene, high levels of *Oct4* allelic pairing should be observed in nuclei of the anterior neuroectoderm where the gene was being coordinately repressed, whereas lower levels of *Oct4* allelic pairing would be expected more posteriorly where *Oct4* is still expressed. Thus, we assessed the frequency of *Oct4* allelic association in sagittal sections of late head fold (LHF)-stage mouse embryos, which were harvested at E7.75 (in collaboration with David-Emlyn Parfit and Michael M. Shen, Columbia University Medical Center). Embryos were immunolabeled with an OCT4 antibody to identify regions of the embryo actively expressing the OCT4 protein, and immunostaining was assessed in three embryonic regions as indicated: anterior, middle, and posterior. Next, multicolor DNA FISH (fluorescence in situ hybridization) was performed, using DNA probes to *Oct4*, *Sox2*, and *Nanog* gene loci. Distances between homologous alleles were measured in ectoderm/neuroectoderm cells in sections taken from three separate E7.75 embryos, in at least 100 individual nuclei per region in each embryo, and the percentage of nuclei with paired homologous loci was calculated for each embryonic region. Interestingly, pairing of homologous *Oct4* alleles was observed at roughly equal levels (~25%–30%) in ectoderm/neuroectoderm cells of all three regions, regardless of the level of OCT4 immunofluorescence observed in each region. When distances between *Nanog* and *Sox2* alleles were assessed in the same nuclei, allelic associations were not observed. Because *Oct4* allelic pairing is observed throughout the ectodermal/neuroectodermal layer at this stage of development, this suggests that *Oct4* allelic pairing occurs in embryonic cells as they are

losing pluripotency and transitioning to a range of differentiated cell lineages in the mouse embryo.

On the basis of these data, we propose a model in which *Oct4* allelic pairing functions to modulate the expression of the *Oct4* gene, thereby facilitating the transition between the pluripotent state and lineage commitment. According to our model, in the pluripotent state, *Oct4* is transcriptionally active and the *Oct4* alleles are separated in nuclear space. Upon induction of differentiation, ESCs enter a transitional state in which the alleles of the *Oct4* gene transiently colocalize in the nucleus, allowing for a transvection-like modulation of *Oct4* gene expression levels as the promoter and enhancer regions of each *Oct4* allele come in close proximity with each other. Once the appropriate *Oct4* expression level has been achieved, the cell may exit the transitional state and progress to lineage commitment.

Monoallelic gene expression describes the transcription from only one of two homologous alleles of a particular gene. We previously performed an unbiased RNA-sequencing screen to identify random monoallelically expressed genes taking advantage of a hybrid ESC line which is an F_1 cross between C57BL/6 and CAST/Ei strains, so that the expressed single-nucleotide polymorphisms (SNPs) would reflect from which allele the transcript is derived. We identified a 5.6-fold increase from just 67–376 genes exhibiting random autosomal monoallelic expression during differentiation of mouse ESCs to neural progenitor cells (NPCs), indicating that monoallelic expression is acquired upon lineage commitment. These 376 genes represented ~3% of expressed genes in NPCs.

During the past year, we examined the impact of monoallelic expression on the transcriptional output of each of these genes. We performed linear regression analysis to compare expression levels of individual monoallelically expressed genes across the independent NPC clones to determine if there was a correlation between the extent of allelic imbalance and total expression level. Using this approach, we identified 30 monoallelically expressed genes (8%) with evidence for transcriptional compensation and 54 genes that followed the dosage of active alleles. The remaining genes either showed intermediate responses or were highly variable and so not able to be confidently classified based on data from six independent clones. We validated the linear regression analysis by quantitative reverse transcriptase–polymerase chain reaction (RT-PCR), confirming transcriptional compensation for seven out of nine genes (78%) and dosage sensitivity for seven out of 11 genes (64%) tested. The identification of transcriptional compensation is intriguing because it suggests that for these genes, the exact level of transcript is more critical than for others. Furthermore, it supports a model in which the biological consequences of monoallelic expression are not limited to reducing transcript levels in the cell, but rather may be a reflection of the stochastic nature of gene regulation at independent alleles.

On the basis of our findings, we propose that random monoallelic expression exemplifies a stochastic aspect of gene regulation that takes place upon the initiation of specific differentiation programs, resulting in global changes in chromatin compaction and gene expression. If the probability of gene activation or repression is less than 1 for a particular gene, this would result in a mixed population of cells containing 0, 1, or 2 active alleles, which, once established and not detrimental to the cell, could be subsequently maintained across cell generations and propagated clonally. In all cases, the two alleles are independently regulated with a low activation probability, possibly due to limiting accessibility of key activating factors. One outcome of this independent regulation is that it results in both monoallelic and biallelic cells in a mixed population. Indeed, at least one biallelic clone is observed for almost all monoallelically expressed genes, consistent with an independent stochastic regulation model. The outcome of monoallelic expression for some genes may be unfavorable if the cell requires a specific level of transcript that cannot be accommodated for by the single active allele, thus resulting in cell death. However, either for those genes for which the exact level of transcript is not critical or for those that are able to compensate transcriptionally, monoallelic expression represents a viable outcome for the cell.

Long noncoding RNAs (lncRNAs) (>200 nucleotides in length) represent a relatively recently studied class of RNAs for which functional insight is currently available for only a few candidates out of potentially thousands. 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. We performed a next-generation sequencing screen of poly(A)$^+$ RNA to identify putative lncRNAs that are differentially expressed in mouse ESCs and NPCs. Our goal is to uncover new mechanisms by which lncRNAs regulate gene expression, differentiation, and/or nuclear organization. We identified 334 lncRNAs expressed in ESCs and NPCs. Of these, 148 were greater than threefold down-regulated and 63 were greater than threefold up-regulated during differentiation toward NPCs. Of these transcripts, ~70% are enriched in the nucleus, indicating potential roles in the regulation of gene expression or nuclear organization. Using stringent criteria, we generated a short list of 50 presently uncharacterized and moderately abundant lncRNAs. We are depleting the levels of these lncRNA in a high-throughput approach using antisense oligonucleotide (ASO) technology. Combined with downstream RNA sequencing, we are generating comprehensive and high-resolution data of global gene expression patterns in the context of aberrant lncRNA expression. We have also begun to characterize these lncRNAs with respect to their expression in embryonic and adult tissues, as well as to examine their subcellular localization by RNA fluorescence in situ hybridization protocols with single-molecule sensitivity. We are also optimizing targeted lncRNA knock-down in the course of early embryonic development to assess the potential impact on normal differentiation processes. Finally, we are working on establishing ESC-derived cell lines that will allow us to track the dynamics of selected candidate lncRNP complexes in living cells, as well as to determine the composition of specific lncRNP complexes at the protein level. Our overall aim is to provide a thorough and comprehensive characterization of individual lncRNAs to dissect functional interactions of these RNAs with nuclear proteins and their mechanisms of action at the molecular level.

Probing the Function of *Malat1*, an Abundant Long Noncoding RNA, That Is Overexpressed in Cancer

G. Arun, S. Diermeier, K.-C. Chang (in collaboration with M. Egeblad, Cold Spring Harbor Laboratory; J.E. Wilkinson, University of Michigan Medical School)

lncRNAs, lacking protein-coding capacity, constitute a huge repertoire of gene regulatory molecules. However, there has been limited genetic evidence to support the function of lncRNAs in vivo. *MALAT1* (metastasis-associated lung adenocarcinoma transcript 1) is among the most abundant nuclear enriched lncRNAs. *MALAT1* up-regulation has been correlated with high metastatic progression and poor prognosis of human breast, lung, and prostate cancer. *MALAT1* is highly conserved, and it exhibits an uncommon 3′-end processing mechanism. In addition to its dysregulation in cancer, its specific nuclear localization and developmental regulation are suggestive of it having a critical biological function.

To characterize the role of *Malat1* in primary luminal B breast cancer and its subsequent metastasis, we have used the MMTV-PyMT mouse mammary tumor model. MMTV-PyMT mice exhibit all stages of the disease from premalignant to distant metastasis. *Malat1* lncRNA was knocked down in MMTV-PyMT mice via subcutaneous administration of ASOs at a dose of 125 mg/kg/wk during a period of 7 weeks, after which animals were sacrificed and primary tumors and lungs were removed for histological analyses. *Malat1*-ASO treatment resulted in ~60% knock down in the primary tumor and a significant reduction in tumor progression rate. Detailed histopathological analysis of *Malat1*-ASO-treated tumors showed an increase in well-differentiated ductular tumors, whereas scrambled-ASO-treated tumors progressed to solid carcinomas. There were structurally normally organized preinvasive lesions present in the *Malat1*-ASO-treated MMTV-PyMT mice, suggesting that *Malat1* function is required for the conversion from the preinvasive to invasive stage. Most interestingly, a marked decrease was observed in the incidence of lung metastases, with a reduction of 70%–80% in the number of metastatic nodules, as compared to animals treated with scrambled ASOs. We are currently intercrossing MMTV-PyMT mice with our *Malat1* knockout mice to develop genetic data to support our ASO knockdown studies.

To characterize the cellular effects of *Malat1* knockdown on cancer cell invasion, we have also generated mammary "organoids" from MMTV-PyMT primary tumors. Tumor organoids embedded in Matrigel were treated with *Malat1*-ASOs, and the effects on cellular behavior were followed for 3 days using spinning disk confocal imaging. In such cultures, *Malat1* loss did not affect cellular viability, but a marked reduction in the invasive properties of the three-dimensional

structures was observed. This in vitro assay now allows us to dissect the molecular mechanisms responsible for the effects of *Malat1* on cancer cell invasive properties. Together, our data indicate that *Malat1* lncRNA represents a promising therapeutic target for treatment of metastatic breast cancer.

A Role for JmjD3/Kdm6B in the Induction of H3K27me3-Independent Genes

M. Hübner, J. Li

The regulation of gene expression is associated with modifications at the amino termini of histones associated with active or silent genes. In particular, silenced genes are often enriched in trimethylated lysine 27 of histone H3 (H3K27me3) and Polycomb group proteins. Two proteins with H3K27me3 demethylase activity have been identified, Jmjd3 and UTX. It has been postulated that one or both of these enzymes are needed to remove the H3K27 methylation mark prior to the activation of silenced genes.

Through live-cell and immunofluorescence imaging in human cancer cells, we identified the localization of the Jmjd3 protein in heterochromatic foci and a soluble nuclear pool. Interestingly, the Jmjd3 protein is recruited rapidly (within 5 min) to an activated gene locus that is not H3K27-methylated and it binds transiently to the promoter region of the induced gene. This led us to investigate whether Jmjd3 has a more general role in transcriptional induction that is independent of its catalytic activity. Analysis of coimmunoprecipitated proteins by mass spectrometry indicated that Jmjd3 is associated with proteins of the MLL4 complex, which is involved in the deposition of the H3K4 trimethylation mark on activated genes. Importantly, small hairpin RNA (shRNA)-mediated depletion of the Jmjd3 protein in human breast cancer cells leads to an attenuation of the induction of estradiol target genes, many of which do not carry the H3K27me3 mark. Through a rescue experiment with Jmjd3 point mutants and protein fragments as well as a small-molecule inhibitor for the catalytic site, we found that the carboxy-terminal zinc-finger domain involved in protein–protein interactions, but not the catalytic activity, is involved in the activation of non-H3K27-methylated genes. A genome-wide RNA-Seq experiment in human breast cancer cells revealed two classes of estradiol target genes: one class of genes whose regulation is independent of Jmjd3 and another class that requires Jmjd3 for full activation. Interestingly, a comparison of these genes with genome-wide chromatin immunoprecipitation sequencing (ChIP-Seq) data revealed that most estradiol target genes are not H3K27 methylated.

These data suggest that in addition to the role of Jmjd3 in the demethylation and activation of H3K27-methylated genes, it also has a role in the induction of non-H3K27-methylated genes. This latter function is mediated by a multifunctional complex consisting of Jmjd3 and proteins of the MLL4 complex, which is involved in chromatin remodeling and transcriptional induction.

Evaluation of the Effect of DNA Repair Pathway Choice on Zinc-Finger Nuclease-Induced Double-Strand Breaks

R.I Kumaran, J. Li

DNA double-strand breaks (DSBs) are the most dangerous class of DNA damage. If DSBs are left unrepaired, they can result in genomic instability or cell death. Therefore, cells have evolved complex DNA-damage response pathways to repair DSBs. In mammalian cells, two major and mechanistically distinct DSB repair pathways are nonhomologous end joining (NHEJ) and homologous recombination (HR). NHEJ and HR can be regulated by multiple factors including cell cycle phase. Although cell cycle regulation of the DSB-repair pathway choice on a single-copy locus is thought to be restricted to G_1 phase for NHEJ and to late S/G_2 phase for HR, it remains unclear for a multicopy locus. We have investigated DSB pathway choice by developing and using multicopy and single-copy DSB reporter cell systems. Earlier, we have demonstrated that enhanced yellow fluorescent protein (EYFP)-Rad51 (HR protein) is recruited at a high frequency (90%) to the multicopy DSB reporter as compared to the single-copy DSB reporter cell line (20%), upon induction of DSBs by green fluorescent protein–zinc-finger nucleases (GFP-ZFNs). This result strongly suggested that donor copy number could be an important determinant that could alter pathway choice toward HR. To test this in the single-copy DSB reporter cell line, which did not have a fluorescent reporter readout for gene correction, we have used a molecular approach, by examining the

sequence alterations at the ZFN-induced DSB. In the absence of donor and upon induction of DSB, NHEJ-induced repair will result in indels at the ZFN target site. However, in the presence of the donor template (which has isogenic codon substitutions, to exhibit a five-nucleotide sequence variation at the mutant ZFN site), when HR repair occurs, the ZFN site in the genome of the reporter cell line will incorporate the five-nucleotide variant sequence. For molecular analysis, the reporter cells were electroporated with ZFNs in the presence/absence of donor or with increasing donor concentrations. Then, genomic DNA was isolated after 24 or 72 h and PCR-amplified to generate a 400-bp product flanking the ZFN target site. The PCR products were barcoded and sequenced using the PacBio SMRT technology. Initial observation of PacBio sequence data indicates that the HR frequency increases with increasing donor copy number.

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

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

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

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 stage, the late viral genes (such as the capsid proteins) are also 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 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 the effects of such phosphorylation events were, we subjected recombinant E1, purified from *Escherichia coli,* to CK2 phosphorylation and tested the protein in various in vitro assays related to initiation of DNA replication. Interestingly, CK2 phosphorylation of E1 resulted in loss of sequence-specific DNA binding. To map the phosphorylation sites responsible for inactivation, we tested the amino-terminal half of E1 ($E1_{1\text{-}308}$), which contains the E1 DNA-binding domain (DBD), for binding with or without phosphorylation. This fragment was also inactivated for DNA binding, demonstrating that the inactivating sites are present in the amino-terminal half of E1. In collaboration with Dr. C. Ruse (CSHL Proteomics facility), we performed mass spectrometric analysis of E1 phosphorylated by CK2 in vitro and identified multiple phosphorylation sites in the amino-terminal domain, each of which was individually capable of inactivating E1 DNA binding. Interestingly, no phosphorylation sites were present in the E1 DBD, demonstrating that phosphorylation events outside the E1 DBD inactivate DNA binding. Consistent with this result, the isolated E1 DBD could not be inactivated by CK2 phosphorylation. Inspired by this result, we next examined the effect of CK2 on the 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 phosphorylation sites responsible for the inactivation of DNA binding are located outside the E2 DBD, demonstrating that the inactivation of the DBD is not caused by direct phosphorylation of the DNA-binding surface.

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 a greatly increased level 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. We believe that this lack of a phenotype indicates that phosphorylation of E1 likely has a role during the vegetative stage of the viral life cycle.

A Dynamic Look at DNA Unwinding by the E1 Replicative Helicase

Hexameric helicases perform functions conserved throughout evolution, including unwinding the DNA double helix during replication. Despite their prominent roles in biology, some of the basic aspects of these helicases, such as whether they use a strand exclusion mechanism or whether they translocate along double-stranded DNA, have been subjects of considerable debate. Viral replicative helicases, such as SV40 large-T antigen (Tag) and papillomavirus E1, have provided the opportunity to study some of these basic features largely because of their homohexameric architecture. These viral helicases form double hexameric (DH) structures on their respective origins of DNA replication and unwind the DNA bidirectionally. It is well established that E1 can form hexamers with helicase activity on single-stranded DNA (ssDNA), and in the structure of the hexameric E1 helicase with ssDNA, the orientation is such that the amino-terminal part of the polypeptide is closest to the 5′ end of the DNA. Given that E1 is a 3′-5′ helicase, this would mean that the hexamers translocate with the amino terminus leading and that the two hexamers have to pass each other in order to unwind DNA. Although this is a very clear result, earlier electron microscopy data from Tag has indicated that perhaps the DH unwinds DNA in a manner fundamentally different from that of the hexamer. These data suggested that the DH encircles double-stranded DNA (dsDNA) and uses a pumping mechanism for unwinding. In collaboration with L. Joshua-Tor at CSHL and Taekjip Ha at the University of Illinois, we set out to distinguish

between these possibilities. We first demonstrated, using fluorescence resonance energy transfer (FRET) analysis with fluorescent labels on the DNA and on the protein, that the orientation of ssDNA relative to E1 is the same in solution as was observed in the E1 structure. Second, we wanted to distinguish between models where one or two strands pass through the DH ring. It has been shown that some hexameric helicases can displace streptavidin bound to a biotinylated template in the process of unwinding. We used this ability to determine whether one or both strands pass through the hexameric ring. We assembled the double trimer (DT) on an ori containing a streptavidin on either the 5′ end or 3′ end. The DT can be converted to a DH, which then unwinds the ori, generating ssDNA. Under these conditions, we could observe displacement of the 5′ streptavidin but not of the 3′ streptavidin, effectively ruling out a mechanism where both strands pass through the central channel of the DH and proving that E1 uses a strand exclusion mechanism for unwinding by both the E1 hexamer and DH.

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DNA REPLICATION AND CHROMATIN INHERITANCE

B. Stillman W. Gao N. Kara S. Ni
M. Hossain A. Mazurek Y.-J. Sheu
J. Jansen S. Nandi P. Wendel

Replication of the genome in eukaryotes has been the focus of this laboratory for 35 years at Cold Spring Harbor Laboratory. During that time, we have progressed from a very superficial understanding of how the DNA double helix within each chromosome is duplicated to detailed insights into both the mechanism and control of this complex process. In particular, studies in recent years have focused on understanding the initiation of DNA replication that is orchestrated by the origin recognition complex (ORC), an ATPase machine that loads other proteins onto DNA prior to actual DNA synthesis. ORC and its associated proteins, the Cdc6 ATPase and the Mcm2-7 hexamer with its chaperone Cdt1, establish a prereplicative complex (pre-RC) at each origin of DNA replication during the G_1 phase of the cell division cycle. We have reconstituted pre-RC assembly in vitro with purified proteins and are now investigating the structure of various complexes that are formed during pre-RC assembly and the next steps that load the DNA polymerases and other proteins that actually replicate the DNA. A key event during pre-RC assembly is the formation of a double hexamer of the Mcm2-7 complex, with each hexamer destined to become part of the active DNA helicase at the DNA replication fork. We have also studied how pre-RC assembly and activation to form two replication forks at each origin are regulated during the cell division cycle.

Dbf4-Cdc7 Control of Pre-RC Assembly and Origin Utilization

During each cell division cycle, DNA synthesis in eukaryotic cells starts from multiple replication origins of DNA replication that are scattered along each chromosome. Replication from each origin only occurs once, thereby ensuring uniform duplication of DNA across the entire genome. The spatial and temporal control of initiation of DNA replication and the subsequent DNA synthesis at replication forks are both highly regulated by a number of protein kinases that ensure genome integrity. The protein kinases include the cyclin-dependent protein kinases cyclin E/CDK2 and cyclin A/CdK2 in human cells and the related Clb5/Cdc28 in yeast. Other investigators have shown that Clb5/Cdc28 phosphorylate two proteins, Sld2 and Sld3, that then bind the Dpb11 protein; somehow this trimeric complex facilitates activation of the Mcm2-7 double hexamer to convert into active DNA helicases.

A second protein kinase, the Dbf4-dependent Cdc7 kinase (DDK), is known to phosphorylate Mcm2-7 hexamer subunits, particularly Mcm4 and Mcm2. Previous studies from this laboratory have shown that DDK binds to a domain within the Mcm4 protein and phosphorylates an unstructured region of Mcm4 near its amino terminus. DDK phosphorylation of Mcm4 is required for the initiation of DNA replication, and removal of the phosphorylation sites in Mcm4 allows yeast to proliferate in the absence of DDK. Thus, the only essential activity of DDK is to inactivate an intrinsic inhibitor of the initiation of DNA replication that resides in the Mcm4 subunit, a part of the Mcm2-7 double-hexamer structure.

In the past year, we have performed a comprehensive analysis of the patterns of origin activation, replication fork progression, and checkpoint responses in cells under replication stress. DNA replication was monitored by pulse labeling nascent DNA strands and then isolating them and sequencing the entire DNA, thereby mapping replicated regions of the genome. The addition of hydroxyurea, an inhibitor of the enzyme ribonucleotide reductase that is essential for production of the dNTP precursors for DNA synthesis, causes replication forks to slow down considerably. At the same time, dNTP depletion triggers Rad53 kinase-mediated checkpoint signaling that then prevents the activation of pre-RCs at origins that have not yet fired (so-called late origins). The studies showed that the Mcm4 amino-terminal

domain, which is intrinsic to the replicative helicase, integrates multiple kinase signaling pathways to control various aspects of the genome duplication process. DDK and CDK both target the structurally disordered amino-terminal serine/threonine-rich domain (NSD) of Mcm4. Using whole-genome replication profile analysis and single-molecule DNA fiber analysis, the results suggested that under replication stress, the temporal pattern of origin activation and DNA replication fork progression were altered in cells with mutations within two separate segments of the Mcm4 NSD. The proximal segment of NSD (closer to the ATPase domain of Mcm4) residing next to the DDK-docking domain mediated repression of late origin firing by checkpoint signals, because in its absence, late origins were activated despite an elevated DNA-damage checkpoint response. In contrast, the distal segment of the NSD at the very amino terminus of Mcm4 had no role in the temporal pattern of origin firing but had a strong influence on replication fork progression and on checkpoint signaling (Fig. 1). Both fork progression and checkpoint response were regulated by the phosphorylation of the canonical CDK sites at the distal NSD. Together, the data suggest that the eukaryotic MCM helicase contains an intrinsic regulatory domain that integrates multiple signals to coordinate origin activation and replication fork progression under stress conditions.

The studies suggest mechanisms by which eukaryotic cells modulate the pattern of replication in response to environmental conditions through the replicative helicase.

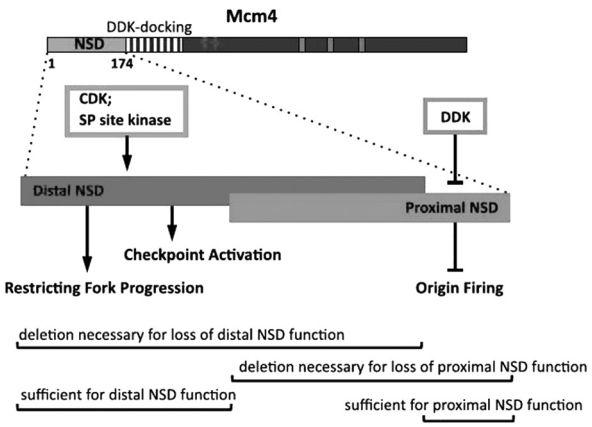

Figure 1. The structure of the Mcm4 subunit of the Mcm2-7 hexameric helicase showing the amino-terminal nonstructured domain (NSD) that is a target for multiple protein kinases that regulate DNA replication.

In other studies related to the function of the ORC and the pre-RC assembly apparatus, we have continued to investigate the structure and function of the ORC subunits in human cells. We have previously shown that the Orc2 and Orc3 subunits localize to the kinetochores of centromeres during mitosis in human cells. More recently, we have shown that both of these subunits form a complex with the BubR1 checkpoint protein kinase that controls the attachment of microtubule spindles to kinetochores. BubR1 is part of a checkpoint process that ensures that the kinetochores of all duplicated sister chromatids are attached to spindles directed to both poles of the mitotic spindles apparatus so that the sister chromatids can be evenly segregated to both poles of the dividing cell. The association of Orc2 and Orc3 with BubR1 requires the protein kinases cyclin B/CDK1 and PLK1, both regulators of progression through mitosis. Current efforts are designed to determine the function of the Orc1 and Orc3 interaction with BubR1.

DDX5, a Regulator of Cell Proliferation in Leukemia Cells

Our laboratory has previously demonstrated that the DDX5 protein is required for the E2F1-dependent expression of DNA replication genes in a subset of epithelial cancers, notably 25% of breast cancer cells. DDX5 was identified via a genetic screen for proteins that are required for DNA replication in epithelial cells. Surprisingly, DDX5 was the only one required in a subset of cancer cells and not in normal cells, and in the cells in which it is essential, the DDX5 gene was either amplified or overexpressed. More recently, in collaboration with Drs. Scott Lowe (formerly at CSHL and now at Memorial Sloan-Kettering Cancer Center) and Chris Vakoc, a series of human and mouse acute myeloid leukemia (AML) cell lines were tested for dependence of AML cell proliferation on DDX5 expression. Depletion of DDX5 caused apoptosis in the leukemia cells (different from the induced senescence in DDX5-sensitive breast cancer cells), and examination of the mechanism revealed the induction of reactive oxygen species (ROS) in these cells. Gene expression (RNA-Seq) analysis, in collaboration with Dr. Jesse Gillis, of the RNA isolated from cells that had either normal

DDX5 expression or DDX5 depletion demonstrated that genes associated with metabolism of glucose were selectively inhibited in the absence of DDX5. Blocking ROS accumulation with the oxygen radical scavenger *N*-acetyl-L-cysteine prevented apoptosis. DDX5-induced apoptosis was also blocked by overexpression of the BCL2 anti-apoptotic regulator, and inhibition of BCL2 with the anticancer drug ABT-737 enhanced the effect of depleting DDX5, suggesting that combination therapy might be a valuable approach to treating chemotherapy-resistant AML.

The results with both epithelial breast cancer cells and AML in mice showed that inhibiting DDX5 might be a good strategy for anticancer therapy, particularly in combination with drugs that enhance apoptosis. The next question asked was how to determine the effect of depleting DDX5 in normal cells in the body—that is, are there any side effects of depleting DDX5 in an adult animal? The reason why this was of concern is that DDX5 knockout mice die during early embryogenesis, but the effect of depletion in adult mice was not determined. Using doxycycline-regulated small hairpin RNAs (shRNAs) that were expressed in all tissues of mice, it was demonstrated that inhibition of DDX5 in normal tissues of the adult mouse did not have any phenotypic consequences. Thus, combination therapy of AML with inhibition of DDX5 may be safe and effective. The pathology analysis of these mice depended on analysis by Dr. John E. Wilkinson, our collaborating pathologist from the University of Michigan Cancer Center.

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CHROMATIN AND TRANSCRIPTIONAL REGULATORY MACHINERIES AS CANCER DEPENDENCIES

C.R. Vakoc A. Bhagwat S. Kawaoka J. Milazzo K. Palacios-Flores C. Shen E. Wang
A. Hohmann F. Mercan J. Minder J. Roe J. Shi Y. Xu

Cancer cells exploit the chromatin regulatory machinery to maintain oncogenic transcriptional programs. This is particularly evident in leukemia, a hematopoietic cancer where genes encoding chromatin regulators often function as driver oncogenes and/or tumor suppressors. Hence, many forms of leukemia can be considered a direct consequence of deregulated chromatin signaling. In our laboratory, we investigate how chromatin regulatory proteins participate in the pathogenesis of cancer.

Mechanistic Studies of Brd4, a Chromatin Reader and Drug Target in Leukemia

F. Mercan, K. Palacios-Flores, J. Roe, C. Shen, J. Shi, Y. Xu [in collaboration with B. Nelson, Cold Spring Harbor Laboratory; R. Young, Massachusetts Institute of Technology, Boston; J. Bradner, Harvard Medical School, Boston]

Original studies from our laboratory in 2011 identified the BET (bromodomain and extra-terminal) bromodomain protein Brd4 as a leukemia dependency and drug target in acute myeloid leukemia. In 2013, Brd4 inhibitors entered Phase-1 clinical trials in leukemia patients. A major focus of our current work is to define the disease-relevant molecular mechanism of Brd4 function in leukemia. We have pursued this question using a host of genetic and biochemical approaches and have made substantial progress in defining a "Brd4 pathway" composed of critical *cis*- and *trans*-acting components that fuel uncontrolled cell growth in leukemia.

One area of focus has been to define the key *cis*-elements used by Brd4 to regulate its important target genes in leukemia cells. Using chromatin immunoprecipitation followed by deep sequencing, we completed a genome-wide survey of Brd4-occupied sites along the leukemia genome. One remarkable observation from these studies has been the realization that a number of Brd4-dependent genes possess large clusters of distal enhancer elements that exhibit profound occupancy of Brd4. These large clusters of enhancers, recently termed superenhancers, appear to be the functionally relevant mode of Brd4-dependent gene regulation. For example, the *Myc* gene is among the most Brd4-dependent in its expression and the *Myc* locus possesses one of the largest Brd4 superenhancers in the leukemia genome. We have performed extensive experiments to assign *Myc* as the relevant target gene of this distal superenhancer, which required chromosome conformation capture technology (3C and 4C). Interestingly, the *Myc* superenhancer closely corresponds to a region found previously as a site of recurrent focal amplification in leukemia patients. These experiments substantially clarify the mechanism of transcriptional regulation performed by Brd4 to support leukemia maintenance.

An additional area of investigation has been to identify the critical lysine acetyltransferases that generate docking sites for Brd4 on chromatin. Through a genetic screening approach, we pinpointed the acetyltransferase enzyme p300 as the critical upstream recruiter of Brd4 at many of its critical target genes. Pharmacological inhibition of p300 catalytic activity results in severe displacement of Brd4 from chromatin. Having pinpointed the catalytic activity of p300 as crucial for Brd4 recruitment to chromatin, ongoing studies aim to determine the critical substrates of p300 that facilitate Brd4 recruitment. On the basis of specific patterns of Brd4 chromatin immunoprecipitation sequencing (ChIP-Seq), we are focusing our analysis on hematopoietic transcription factors as potential acetylated factors that support Brd4 recruitment.

Another area of mechanistic investigation has been the identification of candidate effectors of Brd4 (i.e., interacting partners of Brd4 that facilitate transcriptional activation). Through immunoprecipitation mass-spectrometry analysis, we have found a litany

of Brd4-associated proteins. By filtering of candidates through genetic criteria of having critical roles in leukemia maintenance, we were led to the Nsd3 protein. Nsd3 is a SET-domain-containing histone H3K36 methyltransferase that is not currently well-studied. We found that Nsd3 is recruited to the majority of Brd4-occupied enhancers in the genome. In ongoing studies, we are trying to dissect the molecular mechanism through which Nsd3 promotes Brd4 function at superenhancers.

Currently available small molecules that target Brd4, even those in current clinical trials, lack specificity and inhibit all members of the BET bromodomain family (Brd2, Brd3, and Brdt). Furthermore, these small molecules also lack selectivity among the two Brd4 bromodomains. Hence, it is likely that the therapeutic index of Brd4 inhibitors could be improved upon by achieving a greater degree of selectivity among the BET family. We have sought to explore this question using protein engineering as a means to generate highly selective probes that inhibit individual bromodomains in the BET family. Using phage display, we have generated specific short peptides (selected among random libraries) that can selectivity bind to the bromodomains of Brd4 and can compete with acetyl-histone binding. Remarkably, we have identified specific peptides that can discriminate between the two bromodomains of Brd4. We will continue to develop these approaches to generate highly specific probes that be genetically introduced into cells to define the ideal means of targeting Brd4 in vivo to maximize the therapeutic index of this approach.

Role of the SWI/SNF Chromatin Remodeling Complex in Cancer Pathogenesis

A. Hohmann, J. Shi, J. Milazzo, J. Minder [in collaboration with D. Spector and L. Joshua-Tor, Cold Spring Harbor Laboratory; R. Young, Massachusetts Institute of Technology, Boston]

Cancer cells frequently depend on chromatin regulatory activities to maintain a malignant phenotype. Using nonbiased small hairpin RNA (shRNA) screening of chromatin regulator dependencies in cancer, we have discovered that leukemia cells require the mammalian SWI/SNF chromatin remodeling complex for their survival and aberrant self-renewal potential. Although Brg1, an ATPase subunit of SWI/SNF, is known to suppress tumor formation in several cell types, we found that leukemia cells instead rely on Brg1 to support their oncogenic transcriptional program, which includes *Myc* as one of its key targets. To account for this context-specific function, we discovered a cluster of lineage-specific enhancers located 1.7 Mb downstream from *Myc* that are occupied by SWI/SNF as well as the BET protein Brd4. Brg1 is required at these distal elements to maintain transcription factor occupancy and for long-range chromatin looping interactions with the *Myc* promoter. Notably, these distal *Myc* enhancers coincide with a region that is focally amplified in ~3% of acute myeloid leukemias. Together, these findings define a leukemia maintenance function for SWI/SNF that is linked to enhancer-mediated gene regulation, providing general insights into how cancer cells exploit transcriptional coactivators to maintain oncogenic gene expression programs.

A major unanswered question raised by our findings is how SWI/SNF can have these dual roles both in tumor protection and in tumor formation. We hypothesize that the answer lies in the polymorphic nature of SWI/SNF complexes, which can be formed from a variety of constituent subunits to generate highly diverse complex assemblies. We are currently performing genetic screens that aim to define specific SWI/SNF subunits that are linked to tumor maintenance, but which lack tumor protective functions. We aim to direct our therapeutic approaches to target such subunits in future drug discovery efforts.

A Mediator Complex of Oncogenic Signal Transduction

A. Bhagwat

The major focus of this project is to evaluate the Mediator complex as a therapeutic target in acute myeloid leukemia (AML). Cancer cells are often dependent on aberrant transcriptional programs to maintain their tumorigenic state. Therefore, rationale exists to therapeutically target oncogenic transcription factors. A major obstacle in this pursuit arises from the structure of DNA-binding

transcription factors, which generally lack the deep hydrophobic pockets that are amenable to small-molecule-based inhibition. As an alternate strategy, we have undertaken efforts to target transcriptional coactivators, which have already revealed promising opportunities for drug discovery (e.g., Brd4). To advance this idea, we are evaluating the Mediator complex, a critical coactivator that relays regulatory signals from sequence-specific transcription factors to the core transcriptional machinery. We hypothesize that targeting individual subunits of Mediator will provide a means of interrupting the function of select oncogenic transcription factors. Using a functional genetic approach, we have pinpointed the Med12, Med13, Med23, and Med24 subunits of Mediator as being selectively required for leukemia proliferation, whereas other subunits are more generally required for cell proliferation. This project will focus on evaluating the mechanism of addiction of AML to these Mediator subunits. This will entail cellular, transcriptional, and epigenomic characterization of Mediator function in AML, as well as an in vivo exploration of Med12 function.

Histone H2B Ubiquitin Ligase RNF20 as an Oncogenic Cofactor for MLL Fusion Proteins

E. Wang [in collaboration with R. Roeder, Rockefeller University]

MLL-fusions are potent oncogenes that initiate aggressive forms of acute leukemia. As aberrant transcriptional regulators, MLL-fusion proteins alter gene expression in hematopoietic cells through interactions with the histone H3 lysine 79 (H3K79) methyltransferase DOT1L. Notably, interference with MLL-fusion cofactors such as DOT1L is an emerging therapeutic strategy in this disease. We have identified the histone H2B E3 ubiquitin ligase Rnf20 as an additional chromatin regulator that is necessary for MLL-fusion-mediated leukemogenesis. Suppressing the expression of Rnf20 in diverse models of *MLL*-rearranged leukemia leads to inhibition of cell proliferation, under tissue culture conditions as well as in vivo. Rnf20 knockdown leads to reduced expression of MLL-fusion target genes, including *Hoxa9* and *Meis1*—effects resembling DOT1L inhibition. Using ChIP-Seq, we found that H2B ubiquitination (H2Bub) is enriched in the body of MLL-fusion target genes, correlating with sites of H3K79 methylation and transcription elongation. Furthermore, Rnf20 is required to maintain local levels of H3K79 methylation by DOT1L at *Hoxa9* and *Meis1*. These findings support a model whereby cotranscriptional recruitment of Rnf20 at MLL-fusion target genes leads to amplification of DOT1L-mediated H3K79 methylation, thereby rendering leukemia cells dependent on Rnf20 to maintain their oncogenic transcriptional program.

Chromatin Reader Protein TRIM33 Regulates Cell Survival in Therapy-Resistant Acute Lymphoblastic Leukemia

S. Kawaoka [in collaboration with Y. Suzuki, University of Tokyo]

We have recently extended our epigenetic screening platform into the aggressive blood cancer acute lymphoblastic leukemia of B-cell origin (B-ALL). This disease model is driven by the *BCR-ABL* oncogene and is associated with a dismal prognosis. We evaluated the role of all chromatin regulators in the proliferation of these leukemia cells and have pinpointed a TRIM domain containing protein (TRIM33) in the pathogenesis of this disease. TRIM33 protein acts to support survival of B-ALL cells largely by repressing tissue-specific enhancer function. Notably, knocking down expression of TRIM33 has minimal effects on heterologous cell types. We have recently completed genome-wide studies evaluating chromatin occupancy of TRIM33 in B-ALL and are expanding these studies into other cell lineages. From this work, we hope to gain a general model for how TRIM33 governs leukemia-specific transcriptional regulation.

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See previous page for photos of the following scientific staff.

Row 1: S. Nandi (Stillman Lab); T. Forcier (Wigler Lab); Y.K. Lee, Y. Jiao (Ware Lab); C. Xu (Zhong Lab)

Row 2: J. Li (Spector Lab); A. Stenlund, S. Schuck (Stenlund Lab); D. Gizatullina (Zador Lab); E. Paniagua, J. Regalado (Hammell Lab)

Row 3: D.-W. Hwang (Mills Lab); C. Ardito, D. Olund (Tuveson Lab); P. Deshpande (McCombie Lab); A. Kepecs, P. Masset, J. Sanders (Kepecs Lab)

Row 4: Y. Jacob (Martienssen Lab); S. Ballouz (Gillis Lab); J. Taranda (Osten Lab); K. Liberatore (Lippman Lab)

Row 5: L. Puzis (Sordella Lab); S. Kelly (Huang Lab); A. Churchland, M. Kaufman (Churchland Lab); H.Y. Lee (Schatz Lab)

CANCER: GENETICS

Gregory Hannon is a pioneer in the study of RNA interference (RNAi), a process in which double-stranded RNA molecules induce gene silencing. Hannon and colleagues have elucidated key elements of the RNAi machinery. During the past several years, the Hannon lab has focused on the roles of small RNAs in germ cells, which tend to have the most elaborate set of small RNA pathways of any cell type. They have discovered an essential role for small RNAs, called Piwi-interacting RNAs (piRNAs), that are critical for proper oocyte development and guard the genome against transposable elements. This year, the lab conducted two screens, one in the fruit fly germline and another in somatic cells, to search for new components of the pathway that generates piRNAs. They identified dozens of genes that are required for piRNA production, offering insight into how germ cells ensure genomic integrity. The Hannon lab also strives to understand the biology of cancer cells, with a focus on breast and pancreatic cancer. They 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. These libraries can then be used to identify new therapeutic targets for specific disease subtypes. In addition, they are exploring the roles of small RNAs as oncogenes and tumor suppressors and using genetic approaches to understand the biology of resistance to currently used cancer therapies. Another research thrust of Hannon's team exploits 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, including an emphasis on the evolution of the epigenome and its role in driving cell-fate specification.

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," the Mills group discovered 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. These autism-like movement impairments can be identified just days after birth, suggesting that these features could be used to diagnose autism. Mills has also 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. This year, the Mills lab uncovered how *Chd5* acts as a tumor suppressor: It binds to a protein found within chromatin to turn specific genes on or off, halting cancer progression. 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. They succeeded in halting the growth of malignant tumors by turning on production of one of the proteins encoded by the *p63* gene, called TAp63. TAp63 also exerts other protective effects. This year, the Mills lab generated a mouse model which allowed them to find that TAp63 is required to prevent a genetic disorder, known as EEC (ectrodactyly-ectodermal dysplasia cleft lip/palate syndrome), which is characterized by a cleft palate and major deformities of the skin and limbs in infants. In addition, they recently 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 ways to treat human malignancies in the future.

Scott Powers' work focuses on gene alterations 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 mechanisms by which they are implicated in oncogenesis. They have successfully applied this approach in breast, liver, colon, and lung cancers. Powers has also had an important role in the development of a distinctive CSHL approach to functional study of cancer genes. Called integrative oncogenomics, it is a rapid, large-scale screen for genes that are deleted 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's work provides a new paradigm for understanding and exploring human disease. The Wigler lab studies human cancer and the contribution of new mutation to genetic disorders. The cancer effort (with James Hicks, Alex Krasnitz, and Lloyd Trotman) focuses on breast and prostate cancers. It involves collaborative clinical studies to discover mutational patterns predicting treatment response and outcome and the development of diagnostics to detect cancer cells in bodily fluids such as blood and urine. The major tools are single-cell DNA and RNA analysis. The single-cell methods, which are in development, are also being applied to problems in neurobiology (with Josh Huang and Pavel Osten) to characterize neuronal subtypes, somatic mutation, and monoallelic expression. The Wigler lab's genetic efforts are a collaboration with Ivan Iossifov and Dan Levy, and this team focuses on determining the role of new mutations in pediatric disorders. In a large-scale population sequencing project with W. Richard McCombie and the Genome Sequencing Center at Washington University in St. Louis, and supported by the Simons Foundation, the team has proven the contribution of this mechanism to autism. The work further suggests a relationship between the mutational targets in autism and the process of neuroplasticity that lies at the heart of learning. Smaller-scale population studies of congenital heart disease and pediatric cancer (collaborations with scientists at Columbia University and Memorial Sloan-Kettering Cancer Center, respectively) also point to new mutation as a causal factor in these disorders.

RNA INTERFERENCE MECHANISMS AND APPLICATIONS

G. Hannon

D. Bressan
K. Chang
B. Czech
M. Delas Vives
C. Dos Santos
O. El Demerdash
N. Erard
D. Fagegaltier
I. Falciatori
W.S. Goh
J. Gu
S. Gutierrez Angel
A. Haase
C. Hannon
E. Harrison
E. Hodges
M. Keane
S. Knott
M. Kudla
E. Lee
A. Maceli
M. Mosquera
J. Preall
C. Rebbeck
N. Rozhkov
E. Rozhkova
L. Sabin
S. Sau
E. Seah Jun Wen
M. Soto Ruiz de la Torre
V. Vagin
E. Wagenblast
K. Wasik
Y. Yu
X. Zhou

Our lab continues to focus on three distinct areas. First, we study RNA biology, with a focus on noncoding RNAs. In particular, we are interested in a conserved pathway that protects animal germ cell genomes against parasitic genetic elements. Second, we study the roles of noncoding 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. Historically, we have had a particular focus on using next-generation sequencing to study epigenetic regulation, to identify disease-associated genes, and to help in the establishment of new model organisms.

Fred Rollins left and completed his M.B.A., and Stephanie Shaw and Andres Canela moved on to greener pastures. Assaf (Gordy) Gordon, who had become a fixture in the computational world at CSHL, decided to leave the world of science; however, after a short stint in chilly Canada, he moved back to Boston to work with former student Yaniv Erlich. Antoine Molaro took his special style of science to the West Coast as a postdoc at the Hutch, Poppy Gould moved back to the U.K. as a graduate student, and Yicheng Luo joined former postdoc Alexei Aravin's lab. Marte Andres Terre became a student in sunny California, and we also said good-bye to undergraduates Jorge Ruiz and Jessica Oberheim.

We were joined this year by several undergraduates: Eugene Seah, who will remain as a grad student, and Elvin's Spanish helpers Sara Gutierrez Angel and Mar Soto Ruiz de la Torre, both of whom will join graduate programs in Europe. Jiaqi Gu took on the difficult job of focusing Yang Yu, and Osama El Demerdash stepped in to fill Gordy's very large shoes. A selected set of laboratory projects are described in detail below.

A Novel Technology for the Recovery of Biomolecules

D. Bressan

This past year, I have been in the process of completing the development of a novel technology for the recovery of biomolecules, namely, proteins and their associated nucleic acids, from spatially restricted areas in heterogeneous tissue samples. The method, which we named laserTAG, is based on the interaction between a protein tag fused to a cellular protein of interest and a light-activatable ligand, specific for the tag, that is attached to it only following light irradiation. The ligand is functionalized with a group allowing the affinity purification of the labeled complex. We used this system to perform chromatin pull-down from heterogeneous cell cultures and profile chromatin structure through analysis of the histone-associated DNA. Additional work is currently under way to improve the performance of the technique as well as to develop additional applications.

RNAi as a Path to Target Discovery for Breast Cancer Treatment

K. Chang

Genome-Scale Screening Approach. Breast cancer is a heterogeneous disease that can be clinically categorized into three therapeutic subgroups: estrogen receptor (ER) positive, HER2 amplified, and triple negative (basal-like). The ER-positive (most diverse and numerous) and the Her2-amplified subgroups account for 80%–85% of all human breast cancers, whereas patients diagnosed with the triple-negative subtype have the most aggressive form of the disease and often have poor prognosis. Our goal is to

apply genome-wide, loss-of-function RNA interference (RNAi) screens in cell culture to uncover vulnerabilities of breast cancer cells in all subtypes and discover genes and pathways that modify responses to targeted therapies (lapatinib, trastuzumab, tamoxifen, and estrogen deprivation) for de novo and acquired resistance. We have been using both established and novel preclinical models from collaborators (including Dennis Slamon, University of California, Los Angeles, and Rachel Schiff, Baylor College of Medicine) to conduct RNAi screens in order to identify novel therapeutic targets/drugs that can be translated to the clinic. This study is funded by the Stand Up to Cancer (SU2C) foundation and the NWCRA (National Women's Cancer Research Alliance)/EIF (Entertainment Industry Foundation).

Through a collaborative effort with Dr. Stephen Elledge (Harvard Medical School), we have completed 34 genome-wide screens (28 screens in the Hannon lab and six screens in the Elledge lab) representing all three therapeutic treatment classes. To date, 17 of the 28 CSHL RNAi screens have been deconvoluted (deep sequenced), and sequencing of the remaining screens is ongoing. Various vulnerabilities have been identified for the Her2-positive and hormone-receptor-positive breast cancer subtypes as well as modifiers of trastuzumab resistance.

Focused RNAi Screens Using an In Vivo Approach. Three-dimensional culture systems mimicking the spherical organization of breast epithelial cells recapitulate much of the morphogenetic programs of mammary development and thus are useful tools to address how breast cancer cells survive in a more physiological context than in two-dimensional cell culture. Although these are valuable tools to gain insight into mammary gland biology during normal and cancer development, we aim to study the behavior of breast cancer cells in a more natural environment. To this end, we are developing RNAi screens in vivo using cells derived from human primary tumors engrafted into immunocompromised mice. We have acquired patient tumor-derived xenografts (PDXs) from Dr. Alana Welm (University of Utah) and are expanding these PDXs in vivo to obtain sufficient quantities of tumor cells for in vivo screening. On the basis of collaborative studies with the Dr. Charles Sawyers lab (Memorial Sloan-Kettering Cancer Center), data supports the notion that subpopulations of cells within a tumor could enrich stochastically during tumorigenesis. This poses a problem for RNAi screens in solid tumors by hampering target identification. Thus, we have embarked on a study to gain a deeper understanding of tumor heterogeneity in breast PDX cells by tracking tagged subpopulations of tumor cells during primary tumor establishment and metastasis. This study will provide the basis for developing a more optimized approach to RNAi screening of clinical samples in vivo.

Molecular Characterization of piRNA Biogenesis in *Drosophila*

B. Czech

The Piwi-interacting RNA (piRNA) pathway provides a sophisticated defense mechanism that protects the genetic information of animal germ cells from the deleterious effects of molecular parasites such as transposable elements. This conserved small RNA silencing system consists of Piwi-clade Argonaute proteins and their associated 24–28-nucleotide RNA-binding partners, the piRNAs. piRNAs typically descend from acute transposon mobilization events and from piRNA clusters, which provide a genomic memory of ancestral transposon activity, through the coordinated activity of several processing factors. Although a candidate nuclease, called Zucchini, has been identified, the precise mechanism that converts transposon and cluster transcripts into mature piRNAs remains largely elusive.

We have recently performed a reverse genetic screen that aimed to find all genes that participate in piRNA-mediated genomic defense. Follow-up experiments have revealed a number of previously unknown factors that are crucial for piRNA biogenesis. Through genetic experiments combined with high-throughput sequencing, we identified two proteins, termed GASZ and PIMP, that are essential for the production of piRNAs from cluster transcripts. Interestingly, both proteins share their cellular localization on the outer surface of mitochondria with the presumed nuclease Zucchini. Using genetic, molecular, and biochemical approaches, we are currently dissecting the specific roles of each of these factors individually, as well as their interplay, in order to fully understand the molecular mechanism of the piRNA processing machinery.

Mouse Mammary Glands

C. Dos Santos

During the past few years, we have sought to define the changes in DNA methylation and gene expression that are induced by pregnancy in the mouse mammary gland. We investigated how pregnancy-induced changes control normal mammary gland development and how these modifications could protect from breast cancer development. We have found that pregnancy brings about profound epigenetic remodeling, altering the character of well-defined cell types within the mammary gland. Most relevant is that these epigenetic modifications persist as a long-term memory in the mammary epithelium, even after the signals from pregnancy are long gone. Many of the pregnancy-associated changes in DNA methylation encompass STAT-binding sites. Thus, changes in STAT transcription-factor-regulated gene expression might contribute to long-term changes in cancer risk. We are currently validating genes that presented pregnancy-associated changes at DNA methylation levels and expression levels with regard to their ability to prevent mammary gland cell transformation in vitro and in vivo.

A Role of Germline PIWI Proteins and piRNAs in Cancer

D. Fagegaltier

PIWI proteins are detected in several human and mouse tumors, yet no direct link has been established between cancer and a piRNA response to genome instability. In a STARR Cancer Consortium collaboration, we have derived a large collection of immortalized cell lines from *Drosophila* embryos expressing an activated oncogene or mutant for a tumor suppressor gene. The comparison of their transcriptomes provides a comprehensive view of how genomes coordinate their genetic and epigenetic responses to acquire a transformed state under the stress of specific oncogene and tumor suppressor-activated signaling pathways. We identified cell lines expressing a complete piRNA machinery including Aubergine (AUB) and Argonaute3 (AGO3), proteins known to amplify the piRNA response exclusively in ovarian germ cells. The piRNA pathway in these cell lines is fully functional: Long transcripts derived from the major somatic piRNA cluster flamenco are processed into characteristic piRNAs that eliminate transcripts made from transposable elements (TEs). Similar to the situation in germ cells, piRNA production is abolished, and TE transcripts are affected following the depletion of the major RNA nucleases and cofactors of PIWI (Zucchini or Armitage), but not AUB or AGO3. However PIWI-bound piRNAs present signatures that distinguish these fly cancer cells from both somatic follicle cells (OSS) and germ cells: The piRNAs originate in part from clusters not active in OSS cells, and the bidirectionally transcribed piRNA cluster at 42AB, typical of germ cells, remains inactive. This work suggests the exciting possibility of a functional role for the piRNA pathway in cancer and identifies Ras and Hippo as two major signaling pathways upstream of the response. The in vitro system will be an invaluable tool to characterize the role of the piRNAs in cancer and in the long term may provide a therapeutic target.

Role of Small RNAs in Mouse Spermatogenesis

I. Falciatori

Small RNAs are particularly important during mouse spermatogenesis. Both microRNAs (miRNAs) and piRNAs are expressed in the male germline, and spermatogenesis is defective in any situation in which one of these pathways is affected.

We identified an X-linked miRNA cluster highly and specifically expressed in the male germline. To determine its role during different stages of spermatogenesis, we aim to produce a conditional knockout and several Cre transgenic mouse lines. We engineered a bacterial artificial chromosome (BAC)-targeting construct in which the miRNA cluster is surrounded by LoxP sites. However, our attempts to produce targeted embryonic stem cells (ESCs) by regular homologous recombination have so far failed. We therefore turned to the recently developed CRISPR engineering technology, which harbors the potential to improve targeting efficiency. We are currently trying to target the cluster in mouse ESCs using this technology. We have already produced transgenic lines expressing the Cre recombinase in different stages of germ cell development, and are currently testing the specificity of Cre expression using a fluorescent reporter mouse line. Experiments to test the effect of overexpression of this miRNA cluster in ESCs are also under way.

piRNAs and piwi proteins are part of a defense mechanism that acts during embryonic development to limit the mobility of TEs in the germline. As part of this mechanism, Miwi2 regulates TE expression level by inducing DNA methylation of their promoters. However, the precise molecular and biochemical mechanism by which Miwi2 is able to direct the DNA methylation is currently unknown. Miwi2 is only expressed in germ cells during a very short window of embryonic development, making biochemical studies in this system particularly challenging. To overcome this hurdle, we are taking two different approaches. First, we are trying to develop an in vitro system to produce high numbers of embryonic germ cells by differentiation from mouse or human ESCs. This will allow us not only to have ready access to a high number of embryonic germ cells, but also to use shRNA technologies to pinpoint possible intermediate players in the DNA methylation mechanism. As an alternative strategy, we produced transgenic mouse lines in which a Miwi2-GFP (green fluorescent protein) fusion is expressed under the control of the Miwi promoter. Miwi is expressed starting at meiosis and is associated with a class of piRNAs (meiotic piRNAs) that differ in origin and sequence from those usually associated with miwi2. The ectopic expression of miwi2 in meiotic and postmeiotic germ cells increases the number and accessibility of cells expressing miwi2. It can also give insights into the targeting mechanism for DNA methylation because miwi2 should now be directed to ectopic locations by its association with the meiotic piRNA. So far, for all the lines we produced, the mice look healthy and fertile. We are currently testing the distribution of miwi2-GFP in the different transgenic lines. We will select two different lines to confirm the association with meiotic piRNAs and analyze the genome-wide DNA methylation to establish whether methylation gets introduced in unusual locations under the direction of the meiotic piRNAs.

Asterix Is Essential for Transcriptional Silencing of Transposons in the *Drosophila* Germline

P.M. Guzzardo, F. Muerdter

piRNAs, together with Piwi-clade Argonaute proteins, constitute an evolutionary 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 our current understanding, nuclear Piwi silences transposable elements at the level of transcription by inducing formation of repressive chromatin over transposon loci. However, it is unclear what other factors are involved in this process.

In a genome-wide RNAi screen in *Drosophila* ovarian cells, we identified *asterix*, a gene that is indispensable for repression of endogenous retroelements. In vivo follow-up experiments revealed that knockdown of *asterix* (CG3893) led to elevated transposon transcript levels that were only paralleled by disruption of core components of the piRNA pathway. Nevertheless, mature piRNA populations remained unchanged, hinting toward a specific role in the effector step of the piRNA pathway. Indeed, disruption of *asterix* function leads to loss of repressive histone marks over transposon loci and massive up-regulation of their transcriptional activity. This points toward a central role of *asterix* in the Piwi-associated silencing complex.

Functional Analysis of Ectopic Germline Gene Expression in Cancer

A.D. Haase

Considerable similarities between germ cell development and tumor progression have long been observed: Cancer cells, like germ cells, struggle in a complicated balance between genomic stability and flexibility; both are considered immortal, and analogous to the migration of germ cells, cancer cells metastasize. To ensure genomic stability, germ cells use specialized RNAi pathways that silence transposons. At the core of these pathways are PIWI proteins and their associated small RNAs. Recent studies revealed expression of PIWI pathway components outside of the germline in various cancers, strengthening earlier observations of ectopic germline gene expression in cancer. Such germline genes are potentially ideal targets for diagnosis, therapy, and vaccination because of their restricted physiological expression and unique immunogenic properties. In collaboration with the laboratories of Dr. Gurinder Atwal (CSHL) and Dr. Robert Darnell, we initiated a project with the aim of systematically

investigating ectopic germline signatures and their functions in oncogenesis, through a multidisciplinary approach combining computational, molecular and biomedical methodologies. First, we will assemble a compendium of functional germline gene networks and characterize cancer/germline signatures computationally. Second, we will investigate the function and molecular mechanisms of piRNA pathways in cancer. Based on the importance of small RNA silencing pathways in development and disease and the vital function of piRNA pathways in the germline, we anticipate uncovering a novel layer of gene regulation in cancer. Third, we will elucidate the function of germline signatures in glioblastoma in vivo and evaluate their clinical potential. Overall, this work will provide comprehensive identification and characterization of functional germline signatures in cancer with the aim of identifying candidate vaccine antigens, diagnostic markers, and therapeutic targets.

The Role of miRNAs in Mouse Embryonic Stem Cell Differentiation

M. Kudla

miRNAs are part of the posttranscriptional control mechanism inhibiting protein synthesis. The roles of particular miRNAs in the regulation of developmental processes have been studied extensively; however, the global image encompassing all interactions is still elusive. We focused our efforts on the embryonic stem cell differentiation process in a widely studied model of differentiation toward the neuronal progenitor cells. Thanks to the novel HITS-CLIP (high-throughput sequencing–cross-linking immunoprecipitation) technology applied to our research problem, we were able to obtain snapshots of the miRNAs acting on their transcriptome targets during key differentiation steps. Strict and conservative statistics provides backing to our list of candidate sites located in many embryonic stem cell transcripts. Functional analysis of the gene targets provides evidence for neuronal function-specific miRNA targeting. Validation of the gene list against the external coexpression data set confirms progression of the miRNA targeting from nonspecific to highly brain-specific in neuronal progenitor cells. The unraveled extent of this functional regulation shows the importance of miRNA activity that forms a complex layer of regulation through its network-like effects.

Designation of piRNA Cluster Transcripts in the *Drosophila* Ovary

J. Preall

Transposon silencing in the *Drosophila* germline is a function of a specialized genomic immune system called the piRNA pathway that utilizes small RNAs to program a gene-silencing response targeted at harmful genetic elements. piRNA production is fueled by genomically encoded piRNA clusters that are transcribed and converted into 23–29-nucleotide short RNAs incorporated into PIWI-family proteins. Thus far, three proteins have been implicated in the production of piRNAs specifically from a specialized family of dual-stranded clusters: Rhino, Cutoff, and Deadlock. I have shown that the collapse of dual-strand cluster-derived piRNAs caused by the loss of any of these factors is accompanied by a dramatic decline in the fidelity of the piRNA biogenesis machinery. In these backgrounds, piRNA production is still robust, but it shifts to use aberrant precursors, including a subset of ovarian mRNAs and endo-siRNA loci. In addition, I have identified a novel factor that participates in this process and exhibits phenotypes similar to Rhino, Cutoff, and Deadlock. Cytological evidence suggests that these four proteins function as a complex that sits directly atop piRNA clusters in the nucleus, where they facilitate the channeling of nascent transcripts into the cytoplasmic piRNA production machinery.

The Role of Long Noncoding RNAs in Normal Hematopoiesis and Malignant Transformation

L. Sabin, M.J. Delas Vives

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 and may act by physically recruiting epigenetic modifiers and other protein complexes to target genomic loci.

One developmental process that requires coordinated regulation of gene expression is hematopoiesis. During hematopoiesis, hematopoietic stem cells (HSCs) differentiate to generate both the myeloid and lymphoid lineages of blood cells. 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 feature 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 processes of tumorigenesis, stem cell self-renewal, and differentiation. To this end, we have used high-throughput sequencing of various cell types within the murine hematopoietic lineage. In total, we have performed transcriptome sequencing of 26 samples, which include HSCs, as well as normal and transformed cells from both myeloid and lymphoid lineages.

To identify lncRNAs important in the maintenance or progression of leukemia, we are using a mouse model of AML, which is driven by the oncogene MLL-AF9, a common fusion protein found in human AML. MLL-AF9 leukemia cells can be manipulated and studied in culture, but they can also be injected into recipient animals to induce leukemogenesis. Therefore, we are using short hairpin RNA (shRNA) knockdown technology to determine which candidate lncRNAs have important biological roles in leukemia both in vitro and in vivo. We have verified that lncRNAs can be depleted by shRNA-mediated RNAi and have developed competitive proliferation assays with MLL-AF9 leukemia cells in vitro and in vivo. These assays are amenable to large-scale screening efforts. Therefore, we have compiled a catalog of the lncRNAs that are expressed most abundantly in MLL-AF9 leukemia cells (859 candidates) and are building an shRNA library that individually targets each of these candidates. While the comprehensive shRNA library is being sequence-verified, we are currently performing a smaller-scale in vivo screen of the top 120 highest-priority candidates. Using RNAi knockdowns of candidate lncRNAs in our in vivo competitive proliferation assay, we aim to identify lncRNAs that are important in leukemogenesis and will validate hits both in vitro and in vivo. 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.

In addition to our studies of AML, we also aim to uncover novel lncRNAs involved in HSC self-renewal and differentiation. Despite having been studied for decades, the low abundance and complex physiological niche of HSCs in animals make these stem cells a challenging experimental model. We have established in the lab a unique in vitro culture system that allows us to expand HSCs for several weeks by coculturing them with endothelial cells, one of the essential cell types present in their physiological niche. These HSCs are able to fully reconstitute hematopoiesis when injected into lethally irradiated mice, which provides ultimate proof of their functionality. We can use this coculture system to study the role of lncRNAs in HSC self-renewal and differentiation using an in vitro assay, which reduces the experimental time. The candidate lncRNAs that will be tested in this loss-of-function assay are currently being selected using the transcriptome sequencing data, and we are testing several shRNA delivery vectors to obtain optimal lncRNA knockdown in HSCs. To further characterize the functionality of promising candidates, we can quickly transition to the in vivo model using bone marrow reconstitution assays. We hope to understand how these molecules participate in the regulation of gene expression during hematopoietic regeneration.

RNF17 Prevents Promiscuous Activity of PIWI Proteins in Mouse Testes

V.V. Vagin, K.A. Wasik

PIWI proteins and their associated piRNAs protect germ cells from the activity of mobile genetic elements. Two classes of piRNAs—primary and secondary—are defined by their mechanisms of biogenesis. Primary piRNAs are processed directly from transcripts of piRNA cluster loci, whereas secondary piRNAs are generated in an adaptive amplification loop, termed the ping-pong cycle. In mammals, piRNA populations are dynamic, shifting as male germ cells develop. Embryonic piRNAs consist of both primary and secondary species and are mainly directed toward transposons. In meiotic cells, the piRNA population is transposon-poor and restricted to primary piRNAs

derived from pachytene piRNA clusters. The transition from the embryonic to the adult piRNA pathway is not well understood. Here we show that RNF17 shapes adult meiotic piRNA content by suppressing the production of secondary piRNAs. In the absence of RNF17, ping-pong occurs inappropriately in meiotic cells. Ping-pong initiates piRNA responses not only against transposons, but also against protein-coding genes. Thus, the sterility of RNF17 mutants may be a manifestation of a small RNA-based autoimmune reaction. Our data indicate that RNF17 constitutes one component of a licensing mechanism that prevents deleterious activity of the meiotic piRNA pathway, restricting the loading of PIWI proteins to products of meiotic piRNA clusters.

A Molecular Framework for Understanding DCIS

E. Wagenblast

Ductal carcinoma in situ (DCIS) is the most common type of noninvasive pre-breast cancer in women. It refers to regions of proliferating cancer cells within the milk ducts of the breast and is believed to be the precursor of invasive ductal carcinoma (IDC). The aim of this study is to evaluate the biology of human DCIS through transcriptome sequencing (RNA-Seq). We are using our next-generation sequencing expertise to generate gene expression profiles from single DCIS and IDC lesions and assay them for oncogenic signaling and adaptive immune responses. We are also analyzing the gene expression signature from the surrounding stroma, which may hold the key to determining whether disease progresses to invasive carcinoma.

We have established a collaboration with the Duke SPORE breast tumor bank, which contains core needle samples from 1700 patients including cases of pure DCIS tumors. These tissue samples are cut into 10-µm-thick sections, and individual lesions and stromal compartments are dissected and collected using a laser-capture microscope. Total RNA from dissected individual DCIS lesions are converted to double-stranded cDNA and sequenced using the Illumina next-generation sequencing platform.

We obtained preliminary transcriptomes for ~100 samples derived from normal tissues, stromal tissues, DCIS, and IDC lesions. Each of these samples was prepared from ~50–100 cells. Hierarchical clustering revealed that nearly all DCIS samples in our data set clustered closely together. As we are increasing the number of samples, we are confident that we will be able to identify molecular subtypes of DCIS, including benign DCIS lesions.

Exploring the Functions of *Thermus Thermophilus* Argonaute Protein

X. Zhou

Argonaute proteins are essential components in eukaryotic RNAi pathways. They are also found in a large number of prokaryotic organisms. However, the functions of prokaryotic Argonaute remain unclear. My goal is to understand the function of argonaute protein in the thermophile *Thermus Thermophilus*. In the absence of Ago, plasmid transformation and integration efficiency increases by ~10-fold, suggesting the important role of Ago in defense against foreign genetic invasion. Characterization of small nucleic acids shows global reduction of small RNA, including small RNA from the invading DNA sequence. Interestingly, we observe enrichment of sequences from invading DNA. Besides, another defense system in the CRISPR system, is differentially expressed as the expression level of Ago is altered. Further examination is required to understand the link between Ago and CRISPR.

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TRACKING DOWN CANCER CELL BY CELL

J. Hicks J. Alexander N. Anaparthy H. Cox A. Stepansky
F. Ambrosio T. Baslan M. Riggs

Our laboratory is focused on developing technologies for the rapid molecular profiling of clinical cancer samples, with the primary goal of understanding the cellular complexity of cancer initiation and progression, and through these studies to discover and implement molecular biomarkers into clinical practice. The ultimate goal of this work, the achievement of "precision medicine," will match the right therapies with the right patients at the right time. To this end, our group has established collaborations with oncologists and pathologists at clinical cancer centers across the country in order to apply molecular analysis, in particular, next-generation DNA sequencing (NGS) and copy number variation (CNV) analysis, to profile cancer at its most fundamental level, the single cell.

NGS technologies have revolutionized the study of human cancer genetics. The ability to characterize complete cancer genomes has led to the identification of a rogues' gallery of frequently mutated cancer genes and previously unknown genomic rearrangements, both of which can be used in combination to identify patients for response to new families of targeted cancer therapies. Although many large consortium studies designed to identify the specific mutations in thousands of cancer samples are under way world-wide, our application of NGS has been focused on a related but separate property: the genomic heterogeneity of cancer. Once cancer is initiated, it begins to evolve through rapid and random reorganization of the genes and chromosomes, making each individual cancer a nearly unique disease. Using an NGS technique developed through our close collaboration with Mike Wigler's laboratory (Navin et al., [2011]; Baslan et al., [2012]), we have created the opportunity to track genetic evolution in tumors at the single-cell level and to use genetic markers to track cancer cells as they move through the body. Moreover, we have the opportunity to apply this approach not only to tumors, but also to tissues where only a very small number of cancer cells might be hiding, as in the bloodstream, bone marrow, urine, or lymphatic fluid.

Single-Cell Profiling of Circulating Tumor Cells

Nearly all patients suffering from advanced cancer have a small number of cancer cells in their circulation that are apparently shed from the primary or metastatic sites. These rare "circulating tumor cells" or CTCs can be detected by differential staining for specific protein markers that distinguish them from tens of millions of white blood cells in a typical blood sample. Once detected and isolated, the genetic profiles of these cells provide a novel window into the deep recesses of metastatic cancer and a noninvasive means to understand how cancer evolves and becomes resistant to anticancer drugs in real time in patients undergoing treatment. It is our long-term goal to identify key biomarkers that would direct oncologists to apply the most effective targeted therapy to each patient and to provide an early warning of cancer recurrence or treatment resistance.

In the past year, we have successfully applied our single-cell NGS profiling method to cells isolated in multiple ways from multiple types of cancer. We are now working to make this process faster and cheaper so that it can be widely applied to larger numbers of patients, especially in clinical trials for drug approval. Although one method of simply counting potential CTCs in a blood sample, the Janssen Cell Search process, is currently in clinical use, it has not been possible until now to go beyond enumeration and begin to detect the genetic changes that occur in the cancer during treatment. This year, we have successfully gone beyond enumeration of CTCs by applying of our single-cell profiling technology to genomic profiling of large numbers of CTCs from metastatic breast and prostate cancer patients, comparing the spectrum of genetic events across many cases and following the sequential changes that occur over time in a single patient. This work was done in collaboration with clinical colleagues at Memorial Sloan-Kettering Cancer Center (MSKCC), Scripps Research Institute, and the University of Southern California Cancer Center.

In one project supported by a grant from the STARR Foundation, we sequenced CTCs isolated by fluorescence-activated cell sorting (FACS) from 20 patient samples undergoing treatment by Dr. Howard Scher and colleagues at MSKCC. This work revealed the great differences in genetic makeup of CTCs from patients at different stages of disease and further revealed a novel genetic marker for the onset of resistance to abiraterone, one of the newly approved targeted drugs against metastatic prostate cancer.

In the second project in collaboration with Dr. Peter Kuhn of Scripps Research Institute, we applied our single-cell technology to a very different method of capturing CTCs, where the cells can be identified and phenotyped for protein markers on microscope slides and then stored for many months, after which CTCs can be retrieved one at a time as needed. This method was appropriate for following a single patient through four rounds of therapy and tracking the changes in the genome through that 15-mo time course. Our data showed that during treatment, genetically defined clones of cells could disappear from the bloodstream and new populations of cells could arise within a few weeks of the onset of treatment. After only a few weeks, a third population arose, now with new genetic markers and resistant to the treatment, and the patient suffered a relapse. The rapid time course has put new emphasis on monitoring patients repeatedly through the course of therapy.

The success of these projects has led our group to be recruited, along with Dr. Kuhn, to perform the "translational science" component of an 800-person clinical trial, S1222, for treatment of metastatic breast cancer sponsored by the Southwest Oncology Group (SWOG), a clinical trial organization that CSHL joined in 2011. This trial will open in May 2014.

Profiling Single Cells from Prostate Cancer Biopsies

Another very important clinical issue in prostate cancer is centered on the question of who should be subjected to trans-rectal, multisite biopsies, and furthermore, which patients should have radical prostate removal when cancerous cells are discovered. Both biopsies and prostatectomies carry significant risk to the patient, and therefore, any less invasive means to make such determinations will be of great value to patients and physicians alike. In a joint project with Michael Wigler here at CSHL, we have begun to attack this problem in collaboration with Dr. Herbert Lepor of Langone Medical Center at New York University (NYU). Using our single-cell DNA-profiling methods, we are establishing the "molecular landscape" of early-stage prostate cancer and directly comparing our observations with traditional histopathology on specimens from the same patient.

To retrieve cells from biopsy tissue, our groups jointly devised a nondestructive method in which the freshly extracted biopsy tissue is washed in cell culture medium prior to the biopsy being fixed and treated for standard histopathology. Thousands of cells are exfoliated from the biopsy during the washing step and these cells are then transferred to our laboratory for sorting by FACS, DNA extraction, and single-cell genetic sequencing at CSHL by next-generation DNA sequencing. The biopsy then goes through standard histopathology at NYU. Comparing the results from single-cell sequencing with the clinical Gleason scoring for cancer, we have been able to correlate the progression from benign tissue, showing minimal genetic aberrations, through progression to cancer, with the acquisition of random genetic alterations until one cell begins to replicate rapidly. At that point, corresponding to the Gleason 7 grade by histology, the tissue exhibits a distinct clone of cancer cells that carry the same genetic changes and is clearly cancerous. We therefore believe that "clonality" is a measurable parameter than can be used, in conjunction with standard pathology, as a prognostic marker with enhanced sensitivity for making critical treatment decisions.

These results are consistent with a parallel project being directed by Dr. Wigler (described in his contribution to this Annual Report) on samples taken from radical prostatectomy samples supplied by Dr. Ashutosh Tewari of Weill-Cornell Medical Center.

Single-Cell Profiling of Breast Cancer Biopsies

Building on our earlier results, we have adapted the single-cell sequencing method to a high-throughput platform where we now routinely sequence hundreds of single cells at a relatively low cost. We have since

utilized this platform in a collaborative project with Dr. Lyndsay Harris of Case-Western Reserve Medical Center in Cleveland, as part of a neo-adjuvant clinical trial for the treatment of breast cancer. By profiling hundreds of single cells from pretreated biopsies from all major subtypes of breast cancer (luminal, Her2-amplified, and basal tumors), we are detailing the landscape of tumor heterogeneity across all major breast cancer subtypes. Among the many observations we have made are differing amplifications of driver cancer genes among clonal populations and differing levels of amplification in the same driver oncogene among discrete subclones. Furthermore, for some tumors, we have profiled posttreatment biopsies from the same patients. By analyzing hundreds of single cells from pre- and posttreatment biopsies, we are observing the clonal adaptive response of tumors to therapeutic intervention. Already in some patients, we observed major dynamic restructuring of clonal populations in response to therapy. By relating distinct genomic alterations among sensitive, as opposed to resistant, clones we hope to identify predictive biomarkers of sensitivity to targeted drugs. We are validating our findings using different molecular techniques such as DNA-FISH and exome sequencing of these tumors.

Biomarker Discovery: Retrospective CNV Analysis of Clinical Trial Samples

The DNA copy number method that we used for single cells was originally applied by us to another problematic type of cancer sample: tumor tissue preserved by chemical fixation and embedding in paraffin. This is the type of sample most often available from clinical trials that were completed long enough in the past, often 5–10 years, that there are data available on the patients' outcome. Having outcome data makes these samples valuable for retrospective analysis using newer molecular methods, in order to search for molecular biomarkers that stratify patient groups and identify those that do particularly well or poorly on a specific treatment.

This year, we completed a retrospective analysis of 250 preserved samples from a clinical trial of the drug epirubicin, a member of the anthracycline class of chemotherapy agents, in breast cancer patients. Anthracyclines are effective chemotherapeutic agents in some cases, but they have significant cardiotoxicity, and so identifying a prospective biomarker for those cancers in which the drug will work would have significant clinical value. The BR9601 trial was begun in 1996 and completed in 2008, and preserved samples were provided by Dr. John Bartlett of the Ontario Cancer Research Institute (OCRI) in Toronto. The goal of our work on these samples was to use copy number profiling to locate potential biomarkers for high sensitivity to anthracyclines. Genomic copy number profiling was successful for more than 200 of the samples, and the profiles were analyzed by our collaborator, Dr. Alex Krasnitz, who has developed a novel method for reducing complex genome-wide data to a series of discrete, scorable "cores" of activity. Using this method, Dr. Krasnitz reduced the genome-wide copy number profiles of all of the tumor samples to ~250 major "cores" which were then tested against time to relapse and overall survival data from the trial. Through this method, we have identified a common amplicon of hitherto unknown function, occurring in up to 20% of advanced breast cancers, as a potential biomarker for sensitivity to epirubicin. This project is now in the validation phase with an additional 600 patient samples from a parallel Canadian clinical trial that was undertaken by the OCRI during a similar time period.

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MAMMALIAN FUNCTIONAL GENOMICS

A. Mills Y. Chang D.-W. Hwang Y.-E. Son
G. Horev W. Li A. Vestin

Our research is focused on elucidating the genetic/epigenetic basis of cancer. We have discovered new cancer genes, have revealed mechanisms for how the encoded proteins normally work, and determined what goes wrong during the process of oncogenesis. These findings have had a major impact in the scientific community and have affected how clinicians analyze and treat patients with cancer.

Major discoveries:

- Identifying *p63* as a gene affecting development, aging, and cancer.
- Establishing mouse models of EEC syndrome.
- Identifying TAp63 as a modifier of disease pathology.
- Discovering *CHD5* as a gene that prevents cancer.
- Determining that Chd5's ability to bind histone H3 is essential for tumor suppression.

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

We discovered *p63*, a gene that looked very similar to *p53*—a well-studied gene known to be defective in over half of all human cancers. Besides looking similar to p53, it was not clear how *p63* worked. My group discovered that a lack of *p63* leads to aging. We found that *p63* is needed for stem cell renewal and that when *p63* is depleted, rapid aging features take place, including curvature of the spine, hair loss, and severe skin lesions. Yet, there is a fine balance, as an excess of one version of *p63* (ΔNp63α) causes carcinoma—the most prevalent type of human cancer. In contrast, we found that a different version of *p63* (TAp63) prevents cancer. We showed that TAp63 inhibits tumor growth and that it can do so even when p53 is absent. This work was surprising, as it had always been believed that p53 was absolutely essential for inhibiting cancer. Instead, we found that TAp63 can do the job alone. We are currently working on strategies to turn *p63* on, which might be useful in the clinic.

When we first identified *p63*, we found that it was needed for development, as its loss in mice causes malformations of the limbs, skin, and palate. This work provided a clue to clinicians seeking causes of human syndromes in which children have birth defects including anomalies of the hands and feet, abnormal skin, and severe cleft palate. Our discovery that *p63* is needed for development had a major impact, as it is now known that *p63* mutations cause seven different human syndromes involving birth defects affecting the limbs, skin, and palate. Within the past year, we generated the first mouse models for one of these syndromes, ectrodactyly, ectodermal dysplasia, clefting (EEC) syndrome (Vernersson Lindahl et al. 2013). These mice had anomalies of the limbs, skin (as well as defects in related structures such as hair, tear ducts, sweat glands, nails, and teeth), and cleft palate; these features are analogous to those of children with EEC syndrome. Remarkably, these mice recapitulated a curious feature of EEC that had long been a mystery: Why some children with EEC syndrome have symptoms that are very severe and even life threatening, whereas other children with EEC—even those in the same family that carry the same exact *p63* mutation—have symptoms that are barely noticeable. We discovered a "modifier" gene that controls just how severe the clinical features are in different individuals. We identified TAp63, one of the two major kinds of proteins manufactured from the *p63* gene, as being responsible for the range of severity characteristic of EEC. Mice that have the EEC-causing mutation that also have TAp63 have very subtle defects, whereas those that have the very same EEC-causing mutation but that are also missing TAp63 are very severely affected. This shows that TAp63 normally prevents the adverse effects of the EEC mutation, allowing the fetus to develop quite normally, but when TAp63 is not present, the fetus is not able to develop as it should, resulting in severe EEC features. These EEC models provide functional evidence for the genetic basis of EEC and paved the way for defining TAp63 as a rheostat that

controls EEC-like features in mice. We are currently working to understand how a single-amino-acid mutation such as that found in human EEC perturbs *p63*'s ability to regulate gene expression, leading to abnormal development and cancer.

Discovering *CHD5* as a New Cancer-Preventing Gene

My laboratory discovered *CHD5* as a tumor suppressor mapping to human 1p36—a region of our genomes that is often deleted in cancer cells. 1p36 deletions occur in many different types of human cancers, including those of the epithelia, brain, and blood. Although this suggested that a cancer-suppressing gene resided in this region, its identity remained a mystery. To tackle this problem, my group generated mice with mice with deletions and duplications of the genomic region corresponding to 1p36 using chromosome engineering technology—a strategy with which we can generate precise chromosome rearrangements in the mouse—and we pinpointed a region of the genome with potent tumor suppressive activity. Using 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. Within the past year, we discovered that CHD5 uses its plant homeodomains to bind histone 3 and that this interaction is essential for Chd5 to function as a tumor suppressor (Paul 2013). Our work 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 predicts whether anticancer therapy will be effective; indeed, patients with high levels of *CHD5* have much better overall survival than those with low levels. We are currently delving deeper into the mechanism whereby Chd5-mediated regulation of chromatin affects gene expression cascades that regulate stem cells and cancer.

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CANCER GENES

S. Powers C. Eifert A. Mofunanya K. Revill
J. Li M. Rajaram

My first visit to Cold Spring Harbor Laboratory was in 1980 during the symposium on DNA tumor viruses. I was a graduate student at Columbia and had not left the grime and grit of Manhattan in about 2 years, and so CSHL appeared as an incredible oasis of trees and picturesque buildings and water and brilliant people. At that meeting, a postdoc from Columbia taught me to sail—kind of; however, my first time at the helm I screwed up by sailing into the roped-off area for swimmers and scattered several children. No one came close to being hurt but I was really embarrassed. Just at that moment I saw Jim Watson for the first time. I knew him by sight because I had seen his pictures in the *Double Helix* and *The Eighth Day of Creation*. He was standing on the beach, surveying his domain, seeming quite content, and either oblivious or totally unconcerned about the mild mishap. This made a very favorable impression on me, and it was one of the first of many interesting encounters I've had over the years here at CSHL. The specialness of this place—in addition to its scientific excellence—has induced in me a type of loyalty that other people have for favorite sports teams. I am very grateful to be continuing my association with the Laboratory after I move the base of my operations to Stony Brook in 2014. What follows is a report of projects completed in 2013.

Identification of Tumor Suppressor Genes in Hepatocellular Carcinoma by Genome-Wide Methylation Analysis and Epigenetic Unmasking

K. Revill, J. Li

To identify clinically relevant tumor suppressor genes silenced by DNA methylation in hepatocellular carcinoma (HCC), we integrated DNA methylation data from human primary HCC samples with data on up-regulation of gene expression after epigenetic unmasking. We performed genome-wide methylation analysis of 71 human HCC samples using the Illumina HumanBeadchip27 K array; data were combined with those from microarray analysis of gene reexpression in four liver cancer cell lines after their exposure to reagents that reverse DNA methylation (epigenetic unmasking). We identified 13 candidate tumor suppressor genes. Subsequent validation led us to focus on functionally characterizing two candidates, sphingomyelin phosphodiesterase 3 (SMPD3) and neurofilament, heavy polypeptide (NEFH) that we found to behave as tumor suppressor genes in HCC. Overexpression of SMPD3 and NEFH by stable transfection of inducible constructs into an HCC cell line reduced cell proliferation by 50% and 20%, respectively (SMPD3, $P = .003$ and NEFH, $P = .003$). Conversely, knocking down expression of these genes with short hairpin RNA (shRNA) promoted cell invasion and migration in vitro (SMPD3, $P = .0001$ and NEFH, $P = .022$) and increased their ability to form tumors after subcutaneous injection or orthotopic transplantation into mice, confirming their role as tumor suppressor genes in HCC (Fig. 1). Low levels of SMPD3 were associated with early recurrence of HCC after curative surgery in an independent patient cohort ($P = .001$; hazard ratio = 3.22; 95% confidence interval: 1.6–6.5 in multivariate analysis). Thus, SMPD3 is a potent tumor suppressor gene that could affect tumor aggressiveness; a reduced level of SMPD3 is an independent prognostic factor for early recurrence of HCC.

System-Wide Analysis Reveals a Complex Network of Tumor–Fibroblast Interactions Involved in Tumorigenicity

M. Rajaram, J. Li

Many fibroblast-secreted proteins promote tumorigenicity and several factors secreted by cancer cells have in turn been proposed to induce these proteins. It is not clear whether there are single dominant pathways underlying these interactions or whether they involve multiple pathways acting in parallel. We identified 42 fibroblast-secreted factors induced by breast cancer

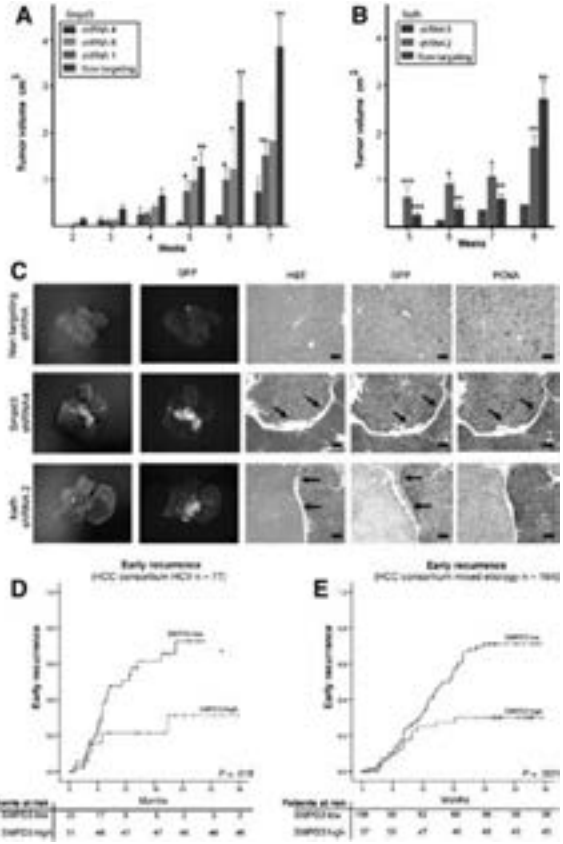

Figure 1. Depletion of Smpd3 and Nefh promotes liver carcinoma formation. (*A*) Subcutaneous growth of tumor protein p53 (p53)$^{-/-}$; Myc hepatoblasts infected with either nontargeting shRNA, shRNA 4, shRNA 6, or shRNA 1 to Smpd3 ($n = 6$ injections; asterisks indicate that the indicated tumor group is significantly different than controls, error bars denote ±S.D., $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$). Tumor volumes were determined from 2 to 7 wk postinjection. (*B*) Subcutaneous growth of p53$^{-/-}$; Myc hepatoblasts infected with either nontargeting shRNA or shRNA2 or shRNA 5 to Nefh ($n = 6$ injections, asterisks indicate that the indicated tumor group is significantly different than controls, error bars denote ±S.D., $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$). Tumor volumes were determined from 5 to 8 wk postinjection. (*C*) Images of mouse livers and sections taken 6 wk after transplantation of p53$^{-/-}$; Myc mouse hepatoblasts with knockdown of either Smpd3 or Nefh. (*Panel columns from left to right*): Intact livers; fluorescent imaging of intact liver for green fluorescent protein (GFP)–positive transplanted cells; H&E staining of liver tissue sections showing the border between normal liver and carcinoma (arrows); immunohistochemical detection of GFP; and immunohistochemical detection of proliferating cell nuclear antigen (PCNA). The last three columns are from the same tissue block. Scale bars: 100 µm. (*D*) Association of SMPD3 expression with time to early and late recurrence after surgery: early recurrence in patients from HCC Genomic Consortium. SMPD3 high-expressing patients are indicated in blue and SMPD3 low-expressing patients are indicated in red. (*E*) Early recurrence in patients from validation cohort. SMPD3 high-expressing patients are indicated in blue and SMPD3 low-expressing patients are indicated in red.

cells using comparative genomic analysis. To determine what fraction was active in promoting tumorigenicity, we chose five representative fibroblast-secreted factors for in vivo analysis. We found that the majority (three out of five) had equally major roles in promoting tumorigenicity, and intriguingly, each one had distinct effects on the tumor microenvironment. Specifically, fibroblast-secreted amphiregulin promoted breast cancer cell survival, whereas the chemokine CCL7 stimulated tumor cell proliferation and CCL2 promoted innate immune cell infiltration and angiogenesis. The other two factors tested had minor (CCL8) or minimally (STC1) significant effects on the ability of fibroblasts to promote tumor growth. The importance of parallel interactions between fibroblasts and cancer cells was tested by simultaneously targeting fibroblast-secreted amphiregulin and the CCL7 receptor on cancer cells, and this was significantly more efficacious than blocking either pathway alone. We further explored the concept of parallel interactions by testing the extent to which induction of critical fibroblast-secreted proteins could be achieved by single, previously identified, factors produced by breast cancer cells. We found that although single factors could induce a subset of genes, even combinations of factors failed to induce the full repertoire of functionally important fibroblast-secreted proteins. Together, these results delineate a complex network of tumor–fibroblast interactions that act in parallel to promote tumorigenicity and suggest that effective antistromal therapeutic strategies will need to be multitargeted.

Two Distinct Categories of Focal Deletions in Cancer Genomes

M. Rajaram, J. Li

One of the key questions about genomic alterations in cancer is whether they are functional in the sense of contributing to the selective advantage of tumor cells. The frequency with which an alteration occurs might reflect its ability to increase cancer cell growth, or alternatively, enhanced instability of a locus may increase the frequency with which it is found to be aberrant in tumors, regardless of oncogenic impact. We addressed this on a genome-wide scale for cancer-associated focal deletions that are known to pinpoint both tumor suppressor genes (tumor suppressors) and unstable loci. On the basis of DNA copy number

analysis of more than 1000 human cancers representing 10 different tumor types, we observed five loci with focal deletion frequencies above 5%, including the *A2BP1* gene at 16p13.3 and the *MACROD2* gene at 20p12.1. However, neither RNA expression nor functional studies support a tumor suppressor role for either gene. Further analyses suggest instead that these are sites of increased genomic instability and that they resemble common fragile sites (CFS). Genome-wide analysis revealed properties of CFS-like recurrent deletions that distinguish them from deletions affecting tumor suppressor genes, including their isolation at specific loci away from other genomic deletion sites, a considerably smaller deletion size, and dispersal throughout the affected locus rather than assembly at a common site of overlap. Additionally, CFS-like deletions have less impact on gene expression and are enriched in cell lines compared to primary tumors. We found that loci affected by CFS-like deletions are often distinct from known common fragile sites. Indeed, we find that each tumor tissue type has its own spectrum of CFS-like deletions and that colon cancers have many more CFS-like deletions than other tumor types.

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CANCER AND HUMAN GENETICS

M. Wigler J. Alexander, J. Allen, P. Andrews, M. Bekritsky, D. Esposito, T. Forcier, E. Grabowska, I. Hakker, R. Kandasamy, J. Kendall, Y.-H. Lee, A. Leotta, B. Ma, S. Marks, J. McIndoo, L. Rodgers, M. Ronemus, J. Rosenbaum, A. Tchaconas, J. Troge, Z. Wang, B. Yamrom, C. Yoon, L. Zhang, Z. Zhu

In collaboration with James Hicks and Alex Krasnitz, among others, we study 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. The lab also develops methodology for single-cell genomic and RNA analysis, 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. 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. In collaboration with Scott Lowe, now at Memorial Sloan-Kettering Cancer Center, we recently showed that tumor suppressor genes are often clustered in large regions that are deleted in common cancers. The single-cell methods are also being applied to problems in neurobiology (with Josh Huang and Pavel Osten) and differentiation (with Chris Vakoc).

Our lab's genetic efforts are a collaboration with Ivan Iossifov and Dan Levy of Quantitative Biology at CSHL and largely focus on determining the role of de novo mutation in pediatric disorders. They participate in a large study of autism organized by the Simons Foundation and also study congenital heart disease and pediatric cancers with collaborators at Columbia University and Memorial Sloan-Kettering Cancer Center, respectively. 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. A large-scale exome sequencing project with our team and the Genome Sequencing Center at Washington University in St. Louis has proven the contribution of small-scale de novo mutations to autism. Overall, results confirm their previous genetic models for autism incidence. In collaboration with Robert Darnell of the Rockefeller University, they have also uncovered a striking link between autism and the genes regulated by the gene that causes fragile-X syndrome, *FMR1*.

The Genetics of Autism

We are examining the genetic basis of autism spectrum disorders (ASD) by studying de novo mutations occurring in affected individuals and comparing rates of different types of mutations with the unaffected siblings. We are in the process of compiling the efforts of three teams studying nearly 2700 families from the Simons Simplex Collection (SSC). Iossifov et al. (*Neuron 74:* 285 [2012]) summarizes earlier findings based on nearly 350 simplex families drawn from the collection, and the major conclusions of that study remain intact. There is a statistically significant increased incidence of de novo mutations of functional consequences (copy number changes, nonsense mutations, splice site mutations, and frameshifts) in affected children compared to siblings, whereas de novo mutations that are likely without effect (synonymous mutation) show no difference between the groups. Mutation incidence is higher in affected girls and increases with the age of the parents. Target analysis indicates a strong overlap of autism candidates with genes expressing mRNAs that are associated with the fragile-X mental retardation protein (FMRP) (collaboration with Robert Darnell at the Rockefeller University). Recurrence analysis suggests that there are on the order of 300–400 target genes. The overall incidence of new mutation, observed and projected, is consistent with new mutation being the major source of autism risk in simplex autism, especially for females.

De Novo Mutation in Sporadic Human Disease

De novo mutations of varying classes have been conclusively linked to neuropsychiatric diseases such as autism, but the overall contribution to sporadic diseases of other types has not been studied in equal depth. To better understand the relationship between rare, de novo copy number variants (CNVs) and sporadic diseases with strong genetic components, we have participated in several smaller-scale studies. In collaboration with Dr. Kenneth Offit of the Memorial Sloan-Kettering Cancer Center, we analyzed copy number variants in 116 families in which a child or young adult was diagnosed with sporadic cancer. Among the patients with testicular cancer, 7% had germline de novo events—a level similar to that seen in autistic individuals and indicating a strong likelihood that de novo mutations underlie at least some types of cancer. Building on this effort, we have begun to perform whole-exome sequencing of these families to detect additional mutations of other classes, such as single-nucleotide variants and small insertions and deletions. The study on copy number variation is now published (Stadler et al., *Am J Hum Genet 91:* 379 [2012]).

Congenital heart disease (CHD) is one of the most common malformations in humans—found in nearly 1% of live births—and is largely sporadic, with evidence of a significant genetic component. Using the same approach as that used in our autism and cancer studies, we studied 213 families in collaboration with Dr. Dorothy Warburton at Columbia University Medical Center. As in the other studies, we found strong evidence that de novo CNVs may cause CHD, with greater than 10% of affected children having a germline variant—a rate five times higher than was seen in a control population. This study is now published (Warburton et al., *Hum Genet 133:* 11 [2014]). These studies suggest that rare de novo mutation contributes too many types of sporadic disorders in humans.

Algorithms for Genomic Analysis

We develop tools for genome analysis by refining existing methods and creating novel algorithms when "off-the-shelf" software is inadequate. Insertions and deletions (indels), as well as other genome rearrangements, contribute significantly to genetic variation and disease. Many small indels can be detected by existing software, but in some genomic contexts—such as within microsatellites—indels can be difficult to discern. To address this need, we have developed our own software. As concrete results, we have identified de novo mutations in microsatellite loci as contributory in perhaps 2%–3% of autism cases. Many of the observations were made using unconventional tools.

Molecular Methods

We have continuously worked on developing methods to perform measurements with greater resolution, or more efficiently, or to make new types of measurements. In the area of genomics, this includes not only algorithm development as described above, but also bench methods. In this regard, we have efforts in three projects. First, we are developing copy number measurements based on sequencing and are driving down experimental costs using sample barcodes, pooled samples, and genome fragmentation. This work is still ongoing. Second, we are developing methods for single-cell DNA and RNA analysis. The latter incorporates the idea of varietal tagging to reduce the distortion caused by polymerase chain reaction (PCR) amplification.

Single-Cell Analysis of Mouse Brain Neurons

Neuronal cells of the brain are the most functionally diverse of any organ in the human body, but the totality of that diversity is not fully understood. Moreover, the diversity of neurons in the brain may be increased by somatic mutational mechanisms occurring during the last few cycles of neuronal differentiation—before the neuronal fate is fixed. With recent advances in single-cell sequencing technology, it is possible to sequence DNA and RNA from single neurons of a given subtype in the brain. In collaboration with Josh Huang and Pavel Osten at CSHL, we are exploring the roles of somatic mutation in mice, categorizing subtypes and specific transcription patterns, and exploring the contribution of monoallelic expression to neuronal diversity and cognitive–behavioral variation.

Genomic Landscape of Prostate Cancer

Prostate cancer (PCa) is the most common genitourinary malignancy among men. It often presents as a multifocal disease characterized by tremendous biologic heterogeneity with a variable clinical course ranging from indolent to lethal disease. Current guidelines for prognostication and treatment strategies rely on clinic pathological parameters such as serum PSA levels and histological appearance. Developing genomic parameters such as copy number variation, genome instability, and clonality will enhance current methods for prognostication and disease management. We are using a single-cell DNA and RNA genomics approach to obtain high-resolution profiles of the genetic alterations that occur in single prostatic cancer cells. Our methods are very general and are applicable to many kinds of cancer and many types of biological sampling, including body fluids.

In collaboration with the laboratory of Dr. Ashutosh Tewari of the Weill-Cornell Medical College and Herbert Lepor at New York University Medical School, we have shown that analysis of the genomes of a few hundred cells can provide a landscape of the evolution of prostatic neoplasia. Comparison to Gleason scores suggests that early stages of the disease comprise cells with unstable genotypes, with little if any clustering into emergent clones with consensus genotypic markers. In contrast, advanced stage disease displays not only cells with divergent and unstable genotypes, but also emergent clones of cells with common chromosomal deletions and amplifications that spread throughout the organ. Efforts are under way to determine whether genome scores derived from single-cell genomic analysis may improve risk stratification in PCa.

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CANCER: SIGNAL TRANSDUCTION

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 the extracellular matrix, and these latter components constitute the microenvironment. Communications between the different components of the tumor influence its growth, its response to therapy, and its ability to metastasize. Among the tumor-associated stromal cells, the lab's main focus is on myeloid-derived immune cells, a diverse group of cells that can enhance angiogenesis and metastasis and suppress the cytotoxic immune response 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 Egeblad lab studies the importance of the myeloid cells using mouse models of breast and pancreatic 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 myeloid cells in tumors during progression or treatment. This technique was instrumental when the lab recently showed that cancer drug therapy can be boosted by altering components of the tumor microenvironments, specifically reducing either matrix metalloproteinases (enzymes secreted by myeloid cells) or chemokine receptors (signal receptors on myeloid cells). This year, the Egeblad lab collaborated with Scott Powers' group to understand how normal cells surrounding a tumor promote cancer growth. They found that normal cells signal to tumors through multiple pathways, and blocking these signals together has the greatest effect on inhibiting tumor growth—offering a new strategy to fight cancer.

Changes in tissue architecture and cell differentiation are often the early signs of cancer, but little is known about the pathways that regulate them. **Senthil Muthuswamy** has developed a novel paradigm for thinking about this aspect of cancer biology. Using sophisticated model systems such as three-dimensional cell culture platforms and transgenic mice, his team found that proteins which regulate cell polarity are involved in both initiation and progression of cancer. Because cell polarity is found to be altered in multiple human cancers, understanding the pathways regulated by them can identify novel molecules and pathways that can be used either as drug targets or as biomarkers for cancer. In addition, Muthuswamy's lab collaborates actively with multiple research teams at CSHL. For example, lab members collaborated with the Mills lab to demonstrate a role for p63 protein in stem cells of the skin and with the Krainer lab to investigate the role that the splicing factor SF2 has in breast cancer. Muthuswamy's lab has ongoing collaborations with the Tonks lab to investigate and identify novel opportunities for targeting tyrosine phosphatases in HER2-positive breast cancers and with the Spector lab to study the role noncoding RNAs have in breast cancer.

Darryl Pappin's lab develops chemical and computational methods for analysis of proteins and peptides. These are fundamental tools for proteomics, and they 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 the results with known or predicted molecules whose amino acid sequences are either known or inferred. Pappin has developed search engines for mass spectrometry data that enable investigators to sift hundreds of thousands of experimental spectra at a time for database matches. He also seeks to reduce sample complexity via an approach he calls chemical sorting. This includes the use of chelation to enrich

phosphopeptides from the total peptide pool and the use of specific affinity-tagged small-molecule inhibitors to segregate classes of kinases or phosphatases for more specific mass spectroscopic analysis.

Despite their large variety of genetic abnormalities, cancer cells have been found to be extremely sensitive to the reversal of certain mutations. **Raffaella Sordella** and colleagues study why cells in certain cancers are responsive to the inhibition of one particular gene or gene product. Why, for instance, do non-small-cell lung cancer (NSCLC) cells that have a particular mutation in the epidermal growth factor receptor (EGFR) respond dramatically to its inhibition by the drug Tarceva? This occurs in 15%–20% of patients, the great majority of whom, within 1–3 years, develop resistance. Various mutations have been implicated in about half of resistant patients. Sordella and colleagues 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 EGFR pathway. The team therefore hypothesizes that inflammation is one of the factors 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 fully characterize the PTP family, understanding how PTP activity is controlled and how PTPs modify signaling pathways. In addition, they are working to determine 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. 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 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 selectively recognize 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 begins at 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 when diagnosed with prostate cancer. Now the team is researching ways to restore the PTEN protein levels in these patients. This therapeutic approach could slow disease progression and thus greatly reduce the need for surgical removal of the prostate or similar drastic interventions that carry the risks of incontinence and impotence. Their second approach to combat prostate cancer is to model the lethal metastatic disease in genetically engineered mice. They are developing a novel

approach that allows for quick generation and visualization of metastatic disease. The efficacy of existing and novel late-stage therapies, such as antihormonal therapy, can then be tested and optimized in these animals. At the same time, the Trotman lab is exploring the genome alterations associated with metastatic disease and with resistance to therapy. To this end, they use single- and multicell genome sequencing techniques developed at CSHL by Drs. Wigler and Hicks.

David Tuveson's lab uses mouse and human tissue models of neoplasia to explore the fundamental biology of these diseases and thereby identify new diagnostic and treatment strategies. His team's main focus is pancreatic cancer, a lethal malignancy that has eluded clinical solutions despite intensive study. The lab's approaches at CSHL run the gamut from designing new model systems of disease to inventing new therapeutic and diagnostic platforms for rapid evaluation in preclinical and clinical settings. For example, they have adopted a new method of culturing tissue fragments indefinitely in cell culture, enabling deep analysis with genetic and pharmacological probes. In addition, therapeutic experiments in mouse models have revealed an important role of redox metabolism and stromal interactions on influencing therapeutic response. This year, they used the mouse model system to identify the mechanism of a promising drug treatment for pancreatic cancer. The lab found that using the drug in combination with more standard chemotherapeutic drugs stopped the tumor growth and lengthened life span for the mice, suggesting that the combination therapy may help overcome the drug resistance that is so commonly found in cancers. Tuveson's lab also has a strong link to clinical trials locally and internationally, with confirmation in early-phase trials the ultimate goal. Collectively, their strategy in the preclinical and clinical arena is codified as the "Cancer Therapeutics Initiative," and this initiative will provide these same approaches to the entire CSHL cancer community.

Linda Van Aelst's lab studies how aberrations in intracellular signaling involving enzymes called small GTPases can result in disease. They are particularly interested in Ras and Rho GTPases, which help control cellular growth, differentiation, and morphogenesis. Alterations affecting Ras and Rho 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, lab members have obtained evidence that OPHN1 has a critical role in mediating mGluR-LTD (long-term depression), 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 into the cellular basis by which mutations in *OPHN1* could contribute to the cognitive deficits observed in patients. Defects in cortical neurogenesis have been associated with cerebral malformations and disorders of cortical organization. The Van Aelst team discovered that interfering with the function of the Rho activator DOCK7 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. In an extension of these studies, the Van Aelst team this year showed that DOCK7 has a central regulatory role in the process that determines how and when a radial glial cell progenitor "decides" to either proliferate, i.e., make more progenitor cells like itself, or give rise to cells that will mature, or "differentiate," into pyramidal neurons. These lines of research provide novel insight into mechanisms that coordinate the maintenance of the neural progenitor pool and neurogenesis.

Hongwu Zheng's lab 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. With the use of 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 (1) to develop various animal models to recapitulate the human glioma pathogenesis and utilize them to trace and investigate in vivo tumor initiation/progression and (2) to identify key pathways/players controlling normal and neoplastic neural progenitor cell renewal and fate determination.

THE INFLUENCE OF THE TUMOR MICROENVIRONMENT ON DRUG RESISTANCE AND METASTASIS

M. Egeblad A. Almeida J. Cappellani M. Fein Y. Kinugasa J. Park J.-H. Park M. Shields R. Wysocki

Solid tumors are aberrant tissues that are composed of cancer cells and stroma. The stroma is the supportive framework of the organs and includes the extracellular matrix (ECM) and fibroblasts, adipocytes, cells of the vascular system, and immune cells. Interactions between epithelium and stroma are essential for normal organ development as well as for tumor progression. In solid tumors, the stroma is also known as the tumor microenvironment.

We study how the tumor microenvironment influences cancer cells in the context of drug resistance and metastasis. We use mouse models of breast and pancreatic cancer together with real-time spinning-disk microscopy in living mice (intravital imaging). This allows us to study how cancer cell proliferation, migration, and survival are influenced by stromal components in real time.

Functional Genomic Approaches Reveal That Multiple, Parallel Interactions Occur between Cancer Cells and Stromal Cells

This work was done in collaboration with Scott Powers at CSHL. Fibroblasts are one of the most prevalent stromal cell types in solid tumors. They have been shown to promote cancer cell proliferation, angiogenesis, ECM remodeling, inflammation, invasion, and metastasis. Tumors containing a high percentage of fibroblasts tend to be higher grade and are associated with poor prognosis.

With Scott Powers, we have undertaken a genome-wide approach to identify important functional genes that are induced in fibroblasts in basal breast cancer. We identified genes up-regulated in fibroblasts upon interaction with breast cancer cells in cocultures and compared the identified genes with those up-regulated in stroma from primary human breast cancers (Fig. 1). Many of the up-regulated genes encode secreted growth factors or cytokines. Using RNA interference (RNAi) with a co-injection tumor formation assay, three of five tested fibroblast-secreted factors were found to have major roles in promoting tumorigenicity. Interestingly, the fibroblast-secreted factors had functionally diverse effects on tumorigenicity, such as stimulation of tumor cell proliferation, inflammatory cell infiltration, and angiogenesis. These results indicate that there likely is no single major mediator of tumor–fibroblast interactions that can be targeted. Instead, there are multiple points of intervention to prevent fibroblasts from supporting breast cancer.

Through intravital imaging, we recently discovered that fibroblasts and breast cancer cells exchange microvesicles. Currently, we are determining the contents of these microvesicles to allow us to compare the effects of tumor–fibroblast communication that occur through microvesicles with those mediated by classical peptide factors.

The Influence of the Tumor Microenvironment on Response to Therapy

R. Wysocki, M. Fein, J. Cappellani

Breast cancer will recur in 20% of patients within 10 years after apparently successful treatment with chemotherapy. When resistance to chemotherapy develops, no other effective treatment options are available. How resistance develops is still an open question, with three main theories proposed for the origin of the resistant cells (1) from subpopulations of cells already within the tumor, (2) from cells with stem-cell-like properties, or (3) through mutations

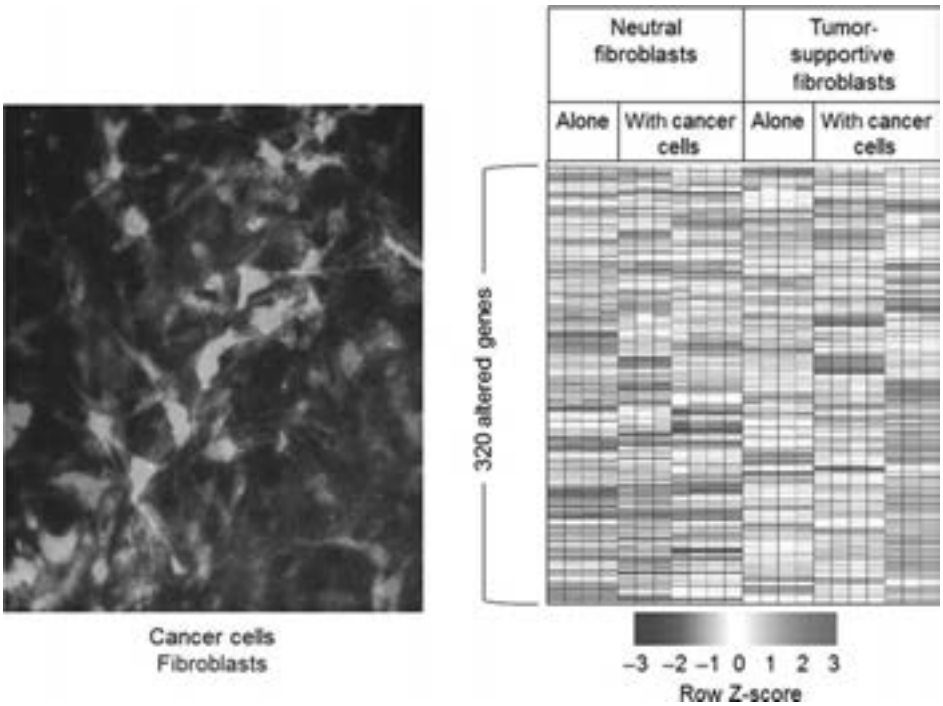

Figure 1. Using imaging and genomics approaches to analyze tumor–stroma interactions. (*Left)* Fibroblasts and cancer cells intermingle in a breast tumor model. A still image (acquired through our new mammary imaging windows) is shown from a tumor in a live mouse. Cancer cells and fibroblasts were co-injected. (*Right*) Gene expression changes induced by culturing fibroblasts with cancer cells lead to promotion of cancer. The separate fluorescent labeling of cancer cells and fibroblasts allowed us to separate the two cell populations and determine how gene expression was changed in fibroblasts after they had been exposed to cancer cells. From the 320 genes identified, five genes coding for proteins that can be pharmacologically targeted were tested for functional relevance, and three of the five genes had major effects on tumor growth.

or epigenetic changes. Understanding which of these mechanisms drive resistance is critical to reducing recurrence.

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 of the responses has been obtained from cell culture or xenograft animal experiments, but such experiments are often not predictive of drug responses in patients. We previously used intravital imaging to show that monocytes are recruited to tumors ~30 h after chemotherapeutic treatment with doxorubicin, after cancer cell death was observed. We determined that monocytes were recruited to tumors through activation of the chemokine receptor CCR2. We further determined that the infiltration of these monocytes contributed to chemoresistance, as the effect of doxorubicin and cisplatin on tumors transplanted to mice lacking CCR2 was significantly better than that of tumors in normal mice.

We noted that when tumors recurred in mice lacking CCR2, they were of lower histological grade than tumors relapsing in wild-type mice. We have now found that the tumors relapsing in mice where host cells lack CCR2 are more differentiated toward a luminal epithelial cell type than the rapidly relapsing tumors in wild-type mice. We are currently investigating how CCR2 expressed by the tumor microenvironment can cause not just a delay in relapse, but also an altered phenotype of the cells that recur. To identify the cellular and molecular mechanisms, we are using lineage tracing with intravital imaging over weeks, using our newly developed mammary imaging windows, together with microgenomics approaches.

Effects of Myeloid Cells on Breast Cancer Metastasis

J. Park, M. Fein, R. Wysocki, Y. Kinugasa

The prognosis of metastatic breast cancer is poor. 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 and that targeting such factors might reduce the ability of cancer cells to take seed. It is now recognized that communication between cancer cells through growth factors and cytokines has an important role in the formation of metastases.

Traditionally, studies of metastasis 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 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 different level of understanding of metastasis might be achieved using technologies that can follow these dynamic processes in vivo.

We use intravital imaging with mouse models of breast cancer and classical genetic manipulation to determine how interactions between cancer cells and stromal cells influence metastasis. We have compared 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 and amount 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 and acts on neutrophils, one of the least-studied inflammatory cell types in cancer metastasis. Ongoing studies are addressing how the chemokine–chemokine receptor signaling axis regulates invasion and promotes metastasis. Preliminary data strongly suggest that cancer cells activate an unusual cell death mechanism in the neutrophils, leading to the release of strong proteolytic enzymes. We have determined that pharmacological inhibitors against both the cell death pathways and proteolysis are effective at reducing neutrophil-promoted invasion in vitro and metastasis in vivo.

Collagen Architecture in Pancreatic Cancer Progression

M. Shields, J. Cappellani

Tumors are often discovered as nodules that are harder than the surrounding tissue. This reflects the changes in ECM stiffening and architecture. The interstitial ECM consists of macromolecules such as fibrillar collagens, fibronectin, and proteoglycans. Type I collagen is the major fibrillar collagen in many tissues and forms a scaffold that provides stability. It also has signaling functions mediated by, for example, integrins and discoidin domain receptors. The synthesis and proteolytic remodeling of the fibrillar type I collagen increases in many tumors, most notably in pancreatic tumors.

In collaboration with Dr. Weaver's lab at the University of California, San Francisco, we previously reported that inhibiting collagen cross-linking in mouse models of mammary carcinoma delays tumor onset and slows tumor progression. We also found that the collagen architectural structure became abnormal with progression of breast cancer: Whereas collagen fibers are curly and oriented in parallel to normal or hyperplastic epithelium, there is a progressive change in the fibers so that they are straighter and mostly perpendicular to the tumor border in the late stages. This changed architecture promoted cell invasion by enabling cells to migrate along the collagen fibers or by enhancing integrin signaling. Enzymes of the lysyl oxidase (LOX) family are important for the collagen cross-linking that results in straighter fibers, and these enzymes have been shown to promote cancer progression and metastasis of breast cancer.

Pancreatic cancer has higher levels of type I collagen and of the LOX-like 2 enzyme than breast cancer, and we therefore hypothesized that collagen cross-linking would have a significant effect on reducing tumor initiation and metastatic spread. However, using both pharmacological inhibitors and genetic approaches, we instead found an increase in the growth of pancreatic tumors and in metastatic spread. Intravital imaging in mice showed that pancreatic cancer cells also migrate along collagen fibers and that collagen architecture is altered by cross-linking inhibitors in pancreatic cancer.

This suggests that collagen architecture or the cancer cells' response to collagen is fundamentally different between breast and pancreatic cancer. Currently, we are determining the effects of the altered collagen architecture on growth, migration, and intracellular signaling.

Employing Tumoricidal Activities of Macrophages

A. Almeida, M. Shields

Tumor-associated macrophages have tumor-supporting activities, and their infiltration is associated with poor patient prognosis. However, macrophages are capable of killing tumor cells 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 breast or pancreatic cancer cells to understand the mechanisms responsible for the tumoricidal activities. We have determined that activated macrophage-mediated cancer cell killing is highly effective and requires direct cell–cell contact between macrophages and cancer cells. A mediator of cancer cell killing has been identified and is under further investigation. Live imaging has also revealed that a small number of cancer cells survive the tumoricidal activities of macrophages. Because such a macrophage-evading mechanism used by cancer cells would hinder therapeutic use of macrophage activation, we used RNA sequencing to identify candidate genes involved in the evasion. Several candidates are under further investigation.

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Mario Shields

PROTEOMICS AND MASS SPECTROMETRY

D. Pappin G. Bencze S. Peacock
C. M. Gauss K. Rivera
T. Hassan J. Wilson

Role of *N*-Acetyltransferases in Neurological Disorders

This work was done in collaboration with M. Doerfel and G. Lyon here at CSHL.

More than 85% of human proteins are acetylated at their amino-terminal amino group, and thus *N*(α)-terminal acetylation is the most abundant posttranslational modification known. Despite its discovery more than 30 years ago, very little is known about the cellular effects or functions of this modification. In humans, six distinct amino-terminal amino-acetyltransferases (NATs) catalyze the transfer of an acetyl group from acetyl-CoA to the *N*(α)-terminal amino group of their specific target proteins.

The major human acetyltransferase, NatA, consists of an auxiliary subunit (Naa15) and a catalytically active subunit (Naa10). Recently, the Lyon laboratory described two families with a lethal X-linked disorder of infancy called the Ogden syndrome. This disorder comprises a distinct combination of an aged appearance, craniofacial anomalies, hypotonia, global developmental delays, cryptorchidism, and cardiac arrhythmias. Using X chromosome exon sequencing and a recently developed variant annotation, analysis, and selection tool (VAAST), the Lyon lab identified a c.109T > C (p.Ser37Pro) variant in Naa10 as the probable disease-causing variant.

To characterize the effects of this mutation, they have generated a yeast knockout NatA cell line and rescue strains ectopically expressing the human Naa15/Naa10 wild type as well as the human Naa15/Naa10 S37P mutant, respectively. Total proteins of these strains were extracted, labeled with distinct iTRAQ reagents, and analyzed by liquid chromatography-mass spectrometry (LC-MS). Analysis of the generated data set revealed specific changes in the expression within ribosomal proteins, indicating an impaired ribosomal function of the NatA knockout and S37P mutants.

Role of Nrf2 in Pancreatic Cancer

This work was done in collaboration with C. Chio and D. Tuveson here at CSHL.

Drug resistance is a major cause of pancreatic cancer lethality, and this has been ascribed to augmented cell survival pathways and poor drug delivery. Nrf2 is a transcription factor that promotes both pancreatic ductal adenocarcinoma (PDAC) progression and drug resistance, and antagonizing the Nrf2 pathway may be clinically advantageous. Because transcription factors are difficult to target therapeutically, the Tuveson laboratory has sought to comprehensively characterize the mechanisms used by Nrf2 to promote PDAC so that more feasible approaches to counter the effects of Nrf2 in PDA may be developed. In pursuit of this goal, we aim to determine the Nrf2-dependent "cysteine proteome" to identify pathways impacting PDA biology and therapy.

Activation of the Nrf2 program is known to elicit a more reducing intracellular environment. Protein oxidation can influence protein activity and stability, and thus, one hypothesis is that certain mediators of Nrf2 function may be "redox sensor" proteins that contain labile cysteine thiol groups. Accordingly, we are using pancreatic ductal organoids to develop a highly sensitive proteomic method that identifies proteins containing cysteines that undergo selective reduction due to the Nrf2 antioxidant program.

We have developed a novel proteomics workflow that uses selective capture of cysteine peptides in conjunction with iTRAQ (isobaric tagging for relative and absolute quantitation) labeling to determine quantitative changes in free (reduced) cysteine residues on a proteome-wide scale that also normalizes these changes to expression levels of the respective proteins. Using this approach, we performed a proof-of-concept experiment using mouse embryonic fibroblasts treated with BSO (buthionine sulfoximine) or DEM (diethyl maleate), two oxidizing agents known to induce Nrf2 target genes; 98% of ICAT (isotope-coded affinity tags)-enriched peptides contained

cysteines, demonstrating the specificity of the ICAT-labeling reagent. We found 349 cysteine-containing peptides modified by both of these treatments from a total of 3662 proteins and 4572 measured cysteine-containing peptides, showing that the approach is effective in quantifying free cysteine changes upon redox alterations. Notably, proteins oxidized by both agents included those previously reported to contain redox sensitive sites, such as Pkm1/2, thioredoxin, and galectin-1. These and other oxidation-sensitive proteins (and the biochemical pathways they impact) will be assessed for the ability to promote PDAC biology and drug resistance, potentially informing new therapeutic strategies.

Oil Production in Duckweed

This work was done in collaboration with S.C. Li and R. Martienssen here at CSHL.

Duckweeds are potential bioenergy crops because of their fast growth and reproduction. Duckweed plants propagate mainly via vegetative reproduction, so maintaining clonal populations is also possible. The Martienssen laboratory aims to increase oil production in duckweeds in order to provide an alternative diesel resource. Because their genome sequencing has already been completed, the target species are *Lemna minor* and *Lemna gibba.* The methods of plant transformation with *L. minor* have also been improved as well as the development of an artificial microRNA system, so genetic engineering for overexpression or knockdown lines is now feasible.

Plant oils are mostly composed of triacylglycerols stored in lipid droplets. Multiple enzymes such as PDAT (phospholipid diacylglycerol) and DGAT (diacylglycerol acyltransferase) are involved in their biosynthesis from glycerols and fatty acids. Overexpression and knockdown duckweed mutants are being developed, both to promote production of specific fatty acids and triacylglycerols and to inhibit degradation to increase overall yield. In addition to these genetic engineering approaches, nutrient stress (such as nitrogen deficiency) has been shown to increase triacylglycerol production in *L. gibba*. To support this project, we have developed a set of quantitative multiple reaction monitoring (MRM) assays to measure several key triacyl- and diacylglycerol species, allowing for rapid screening of lipid composition. Using this technique, we will be able to screen different mutant and stressed plant samples for high accumulation of target triacylglycerols.

Glycopeptide Markers for Pancreatic Cancer

This work was done in collaboration with D. Engle and D. Tuveson here at CSHL.

Pancreatic cancer is the most lethal of the common carcinomas, and by the time it is detected, the disease has usually disseminated to multiple sites. Pancreatic ductal adenocarcinoma (PDAC) presents insidiously with nonspecific symptoms such as vague abdominal discomfort and weight loss, and as a result, it is often not recognized as a developing cancer. But, although this disease is often diagnosed late, its long latency presents an opportunity for early detection and intervention. Ideally, a simple blood test would be able to identify patients at risk of developing cancer, diagnose and stage the type of cancer, and monitor disease progression and treatment efficacy.

Pancreas cancer burden can be followed longitudinally in many patients by measuring the level of circulating tumor antigen CA19-9; however, this biomarker is insufficient for the early diagnosis of pancreas cancer (PDAC) because it lacks the necessary sensitivity and specificity. Thus, there is currently a great need for effective diagnostic tools that enable early detection and quantitative measurement of disease progression. The Tuveson group has produced accurate mouse models of early and advanced PDAC that have accelerated basic and applied research for this disease. Unfortunately, the evaluation of novel diagnostics in mouse models has been hampered by the lack of CA19-9 production in mice due to the absence of certain enzymes that catalyze the production of sialylated Lewis A antigens. The Tuveson laboratory is attempting to generate the first GEMM of pancreas cancer that produces CA19-9 antigens, enabling facile and high-throughput discovery, evaluation, and validation of new diagnostics. This will provide an important preclinical tool for the rigorous analysis of candidate biomarkers before the onset of clinical trials.

We performed immunoprecipitation of CA19-9-labeled proteins that are secreted or shed in monolayer cultures of mouse PDAC cell lines engineered to produce CA19-9. The immunoprecipitated proteins were labeled with amine reactive isobaric mass tags (iTRAQ), and proteins that were present in higher abundance in

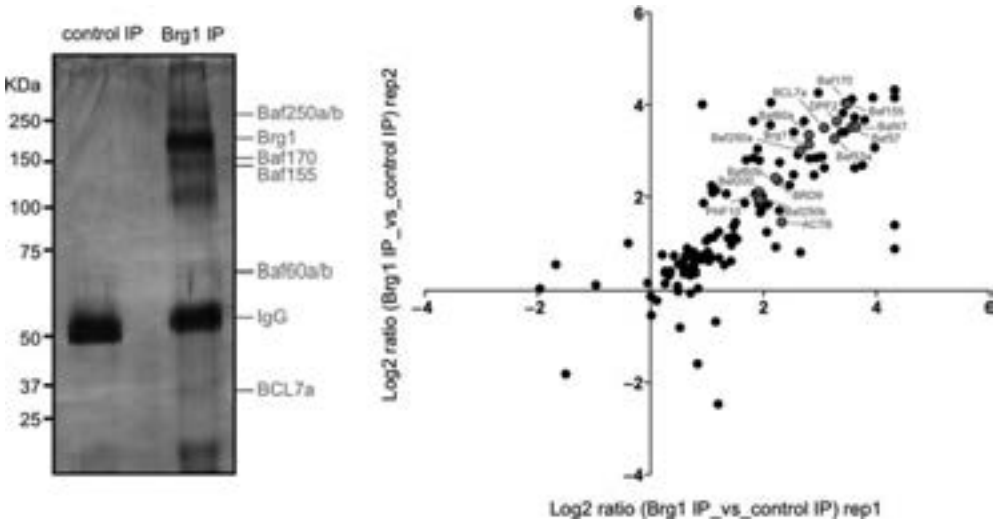

Figure 1. (*Left*) Silver staining of endogenous BAF complex in human leukemia cell line (NOMO-1) that was subjected to iTRAQ mass spectrometry. (*Right*) Two biological replicates of the iTRAQ data quantitatively comparing proteins enriched from Brg1 IP and control IgG IP in human leukemia cell line.

the CA19-9-positive samples were identified by LC-MS. We confirmed the pulldown of known CA19-9-bearing proteins, including Mucin 1 (western blot) and Mucin 5ac (IP/mass spectrometry), as well as other proteins that have known *O*-glycosylation sites but that had not been previously annotated as CA19-9 carriers in human cells. Upon transplantation of these cell lines into the pancreas of C57BL/6 J mice, CA19-9 was detected in the tumors and metastases from the cell lines engineered to produce CA19-9, whereas none were detected in the control, pBabe-neo tumors. Furthermore, high levels of CA19-9 were detected in both the plasma and ascites of mice inoculated with CA19-9-positive human and mouse cells. Using these plasma and ascites samples, we have further optimized the immunoprecipitation of CA19-9 carrier proteins for LC-MS analysis and have identified the known carrier of CA19-9 (Mucin 1) in the ascites of mice transplanted with mouse PDAC cells. Mass spectrometric analyses of the transplants with human cell lines is currently ongoing.

Characterization of BAF Complexes

This work was done in collaboration with J. Shi and C. Vakoc here at CSHL.

ATP-dependent remodeling enzymes are a major category of chromatin regulators that regulate transcription programs underlying a variety of biological processes. The SWI/SNF complex (also known as BAF in mammals) is one of the most studied. In mammalian cells, BAF complexes are composed of ~11 subunits that are encoded by ~19 distinct genes, and the diversity of configuration of BAF subunit assemblies is thought to confer distinct, specialized functions. Recently, the core catalytic subunit of the BAF complex, Brg1, has been shown to have a critical role in leukemia maintenance through sustained expression of oncogenic Myc. However, little is known about BAF complex assemblies in leukemia cells. To investigate the BAF complex in leukemia, we immunoprecipitated endogenous Brg1 to pull down BAF complexes from human leukemia cell lines (NOMO-1) and quantified the protein contents by iTRAQ LC-MS (Fig. 1) We identified numerous Brg1-associated proteins, which included almost all well-known BAF subunits, and some additional protein factors. Currently, we are using immunoprecipitation (IP)-western blotting to confirm the MS results and trying to pinpoint the leukemia-biology functional relevance of these Brg1-associated proteins through genetic and biochemical experiments.

Discovery and Characterization of a Lys/Arg-N Protease

D. Pappin, J. Wilson

Directed peptide fragmentation can generate specific ion fragment series that simplify MS/MS spectra. This is achieved by controlling where the charge is

located on the peptide, either enzymatically or, with extra steps, by chemical modification. To produce predominantly amino-terminal ions, the charge must be placed at or near the amino termini of peptides. This can be most elegantly achieved using a proteolytic enzyme with amino-terminal cleavage specificity at basic amino acids. We have discovered a novel thermostable protease with amino-terminal arginine and lysine specificity. The enzyme can be used as a substitute for trypsin, generates predominantly amino-terminal ions, and completes digestions in ≤1 h at 60°C.

The enzyme exhibits a temperature optimum of ~60°C and a broad pH specificity around neutral. The protease is most active in low ionic strength and MS compatible acetate buffers, and activity requires calcium and zinc. In contrast to trypsin, MS compatible or selectively cleavable detergents did not increase the number of identified peptides or proteins. Specificity for amino-terminal cleavage at arginine and lysine was ≥95%, with the remaining 5% amino-terminal to larger aliphatic residues. In whole-cell lysates, the numbers of identified peptides and proteins were similar to trypsin, yet generated in nearly 20× shorter digestion times. As expected, the b-ion series in MS/MS spectra were significantly more intense than y ions. In complex mixtures, a shift to lower peptide charge states was also observed, likely due to close physical proximity of basic centers. This preference was abrogated by modification with iTRAQ, probably due to the increased distance between positive charges. The efficiency of iTRAQ labeling was unchanged.

This highly active metalloprotease is suitable for extremely rapid (≤1 h) digestion of samples for immediate MS analysis. The peptides produced by this enzyme always have major basic centers at the peptide amino termini, producing more easily interpretable MS/MS fragmentation spectra by generating a dominant b-ion series. In contrast to trypsin, the ion current is not diluted between b and y ions, thus simultaneously increasing detection sensitivity. The speed, specificity, ease of use, and overall effectiveness of this protease, combined with its ability to provide more interpretable spectra, make it a valuable component of the proteomics toolkit.

PUBLICATION

Ruse CI, Peacock S, Ghiban C, Rivera K, Pappin DJ, Leopold P. 2013. A tool to evaluate correspondence between extraction ion chromatographic peaks and peptide-spectrum matches in shotgun proteomics experiments. *Proteomics* **16:** 2386–2397.

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

R. Sordella M. Camiolo M. Pineda
P. Debjani S. Senturk
V. Himenez N. Shirole
T. Lindsted Z. Yao

Recent technological advances in genomic and sequencing technologies have revolutionized the way in which we understand tumorigenesis and have led to the identification of cancer mutations with diagnostic, prognostic, or therapeutic implications. The use of drugs that precisely target such mutations has shown a definitive impact in the management and treatment of cancer patients. It is nonetheless evident that at present, major obstacles still need to be overcome in order to achieve the goal of developing truly curative therapies. Among these hurdles, drug resistance continues to be a major factor in reducing the effectiveness of cancer treatments at large.

Cancer was first described as an evolutionary process by Nowell, who hypothesized that natural selection occurs in tumors in the form of clonal selection leading to constant evolutionary change and possibly to drug resistance. Evolutionary processes in cancer development and progression have since been studied, which has provided remarkable insights into the biology of cancer. It has now become clear that by the time a cancer is detected, it is composed of billions of malignant cells. Although all share somatic mutations that were present in the founder cell, they also carry additional mutations acquired during the natural evolution of the tumor. Thus, although most cancers are monoclonal at their origin, the acquisition of additional mutations and the expansion of the population size result in a dramatic increase in genetic tumor heterogeneity. Somatic mutation analyses of tumors and phylogenetic trees constructed from these data provided evidence for the Darwinian evolution model based on positive selection. As Darwinian evolution is fueled by this population heterogeneity, the study of the origin and the measurement of the extent of genetic heterogeneity are key steps to gaining an understanding of how cancer drug resistance develops.

NRSF Modulates Cancer Cell-State Plasticity by Epigenetically Regulating miR-335 Expression

In recent studies, we found that in addition to the acquisition of genetic mutations, epigenetic changes can also contribute to the observed intramural tumor heterogeneity. In particular, we observed a critical role for NRSF (neuron-restrictive silencer factor) and miR-335 in epigenetic reprogramming of cells. Our data indicate that NRSF and miR-335 can act as "pegs and guy ropes" to shape the cell epigenetic landscape, and by doing so, they can contribute to restraining epithelial cell differentiation and promoting the acquisition of a cell state characterized by the acquisition of mesenchymal-like features, increased metastatic potential, expression of a specific immunotype ($CD44^+/CD24^-$), and distinct drug sensitivity.

This conclusion is based on multiple observations. First, miR-335 expression is significantly decreased in $CD44^+/CD24^-$ tumor cells, and its inhibition in $CD44^+/CD24^-$ cells was sufficient to activate a composite program that resulted in the acquisition of molecular and phenotypic features typical of a $CD44^+/CD24^-$ cell state (i.e., decrease in features associated with epithelial differentiation, acquisition of a specific immunotype, and increased resistance to EGFR-mediated signaling). Second, miR-335 expression is regulated by NRSF recruitment to the MEST (mesoderm-specific transcript) isoform-2 promoter. Inhibition of NRSF expression caused a decrease in DNA methylation of the MEST isoform-2 promoter and rapid reactivation of MEST and miR-335 expression. This was accompanied by decreased expression of genes characteristic of a $CD44^+/CD24^-$ state such as transforming growth factor-β (TGF-β), interferon-6 (IL-6), WNT5a, ECAD (E-cadherin), and vimentin.

miR-335 and NRSF are ideal players for coupling epigenetic inheritance and dynamic interconversion among cell states. Expression of miR-335 can swiftly activate complex molecular circuitries and limit fluctuation among cell states by restricting the expression of a plethora of genes controlling traits that typify the $CD44^+/CD24^-$ cells (i.e., TGF-β, IL-6, WNT-5a, Sox9, CD44, CD24, ECAD, and vimentin). Of particular interest is the regulation of TGF-β, IL-6, and WNT-5a by miR-335 as these cytokines have been shown to be necessary and sufficient to induce and maintain a $CD44^+/CD24^-$ state.

miR-335 can also regulate multiple miRNAs through silencing of TGF-β-mediated signaling: Many of these miRNAs have been associated with key aspects of $CD44^+/CD24^-$ cells such as self-renewal, metastatic capacity, cell reprogramming, and drug resistance. For example, miR-200c and miR-183 clusters are down-regulated in human and murine breast cancer stem cells and in mammary gland stem cells. miR-200c has also been shown to restrain epithelial-to-mesenchymal transition (EMT) by mediating the degradation of ZEB1. Moreover, miR-34a, miR-92a, miR-99, and miR-192 expression is negatively correlated with an increased risk of metastasis. Interestingly, miR-34a was recently shown to be involved in the reprogramming of somatic cells and in the inhibition of growth and metastasis of prostate cancer stem cells by directly repressing CD44 expression.

NRSF, on the other hand, is a well-characterized transcriptional repressor involved in heterochromatin-mediated silencing and in restriction of cell-fate determination. By interacting with corepressors such as co-REST and Sin3a, NRSF mediates several histone modifications that are part of a "histone code" thought to be essential in gene silencing. These modifications include H3K9 trimethylation, a histone modification associated with heterochromatin structures that poises promoters for DNA methylation.

Intriguingly, NRSF is best known for its roles in repressing neuronal gene expression and restricting neurogenesis in nonneuronal tissues and in undifferentiated neuronal progenitors. Hence, the observation that NRSF also regulates the $CD44^+/CD24^-$ cell-state transition supports a broader role of NRSF in cell-fate determination and in maintaining an undifferentiated state that is not restricted to neuronal differentiation.

Our studies also imply that the interconversion among cell states relies on a few events. As a consequence, the system can be easily perturbed not only by inter- and intracellular signals, but also by nondeterministic distribution of Prickle1/NRSF/miR-335 activity. This is particularly interesting in light of recent work by Gupta et al. suggesting that the capability of cancer cell populations to retain phenotypic equilibrium over extended periods of time can be described through the stochastic interconversion of cancer cell states. Hence, in principle, stochastic differences in NRSF binding and/or Prickle1/miR-335 expression could explain the nondeterministic cell transition between cell states.

The dependence of the $CD44^+/CD24^-$ cell state on a nearly singular perturbation can, in principle, also increase cell-state plasticity and, hence, cellular adaptability to environmental changes. This can be of extreme importance in the case of drug treatment. As a corollary, we anticipate that as opposed to anticancer therapies that exclusively target one cell population in tumors, the use of a combination of agents that are selectively toxic to multiple cell states (e.g., EGFR and IL-6) or the concomitant use of these drugs with agents that can reshape the epigenetic landscape, for example, by blocking cells in defined cell states or by shifting drug-resistant cells into a drug-sensitive state, will be more effective.

$CD44^+/CD24^-$ Cells Are Characterized by Deficient Homology-Directed Repair and Increased Adaptability Triggered by TGF-β Down-Regulation of DNA Damage-Response Genes

The emergence of RNA interference (RNAi) as a mechanism to silence gene expression has enabled loss-of-function analysis in mammalian cells in a potentially genome-wide manner. We have utilized such an RNAi-based, forward genetic approach to identify genes that are selectively required for the survival of $CD44^+/CD24^-$ cells. We identified 135 shRNAs that target genes that are selectively required for the survival of $CD44^+/CD24^-$ cells. Strikingly, among them, we also observed shRNAs silencing genes involved in DNA repair/replication pathways. In follow-up studies, we found that the selective sensitivity of these cells

to the inactivation of this particular class of genes was due to a decreased expression of genes that play a critical role in homology-direct DNA repair that was dependent upon the activation of TGF-β signaling. This observation is of particular interest because in addition to sensitizing cells to inactivation of DNA repair pathway components, a decreased expression of genes involved in DDR could also lead to an increased rate of DNA double-strand breaks (DSBs). Consistent with this idea, we observed an increased number of γH2AX-foci-positive cells and larger mean comet tail movement in $CD44^+/CD24^-$ cells sorted from A549 compared to cells with any other immune type and parental cells, as well as in H1650-M3 cells compared to H1650 cells.

In 1943, Luria and Delbrück showed that genetic diversity generated as a consequence of random mutations in each generation of bacteria resulted in a subpopulation that was able to survive T1 phage infection. Thus, in a similar fashion, the defects in DDR that we observed in $CD44^+/CD24^-$ cells could result in an increased phenotypic diversity of cells. Although intratumor heterogeneity is most likely not critical when growth conditions are stable, it could have an important role in the survival and adaptability of a cell population upon drastic environmental changes. In particular, similar to the case of the fluctuation experiment of Luria and Delbrück, it could contribute to acquired resistance upon drug treatment. Indeed, our data reveal that the transition into a cell state characterized by a $CD44^+/CD24^-$ immune type can result in a greater phenotypic diversity and adaptability.

The observed inverse correlation between the expression of these genes and TGF-β in tumors indicates that our findings could be highly relevant to the human disease. In principle, they could explain why tumors characterized by a high content of $CD44^+/CD24^-$ cells, although initially sensitive to drug treatment, rapidly become drug resistant. In addition, because TGF-β is one of the main cytokines produced during inflammation, our data could provide a novel nexus between inflammatory conditions, tumorigenesis (tumor initiation and progression), and acquired drug resistance. The observation that the $CD44^+/CD24^-$ state is particularly sensitive to the inactivation of BRCA1, NEK9, ORC5L, RFC3, POLS, ERCC8, and RPA2 also points to novel actionable drug targets for the treatment of tumors characterized by this phenotype.

One interesting aspect of $CD44^+/CD24^-$ cells is their transient nature. In fact, it has been shown that they can be generated by stochastic fluctuation and/or exposure to paracrine TGF-β. Given that cells in a $CD44^+/CD24^-$ state are intrinsically more genetically unstable, our model predicts a "quanta" evolution of tumors. Fascinatingly, although it was initially assumed that all tumors evolve in a linear mode, recent observations in prostate cancers indicate that certain tumors also progress in a punctuate manner.

On the other hand, defects in the expression of DDR genes have been shown to induce DNA damage responses that could result in cell cycle arrest and/or senescence/apoptosis. The tumor suppressor TP53, often referred to as the "guardian of the genome," has a major role in coordinating DNA-damage responses in cells. Thus, the fact that p53 tumor suppressor activities are often disabled in $CD44^+/CD24^-$ suggests the intriguing possibility that inactivation of p53 is a cardinal feature that empowers TGF-β to accelerate genetic instability and cancer evolution.

P53Ψ Is a Transcriptionally Inactive P53 Isoform Able to Reprogram Cells toward a Metastatic-Like State

$CD44^+/CD24^-$ cells can also be generated stochastically/epigenetically in cells bearing a tp53 wild-type allele, implying that the p53 network in $CD44^+/CD24^-$ cells must be compromised. Interestingly, we uncovered in these cells a novel mode of p53 regulation that involves alternative splicing of the *TP53* gene. We found that the use of an alternative 3′ splice site in intron 6 generates a previously uncharacterized p53 isoform that we named p53Ψ. Intriguingly, this isoform is highly expressed in cells characterized by a $CD44^+/CD24^-$ immune type. At the molecular level, p53Ψ lacks major portions of the DNA binding domain, the nuclear-localization sequence, and the tetramerization domain, features that are normally present in full-length p53 (p53FL). Consequently, this isoform proved to be incapable of sequence-specific DNA binding and *trans*-activation of canonical p53 target genes. However, expression of the p53Ψ isoform attenuated the expression of E-cadherin, induced expression of markers associated with EMT, and enhanced the motility and invasive capacity of normal and malignant cells.

Consistent with a role of these features in enhancing the prometastatic capabilities of cells, we observed that in patients with early-stage NSCLC, expression of p53Ψ correlated with increased probability of relapse following surgical tumor resection. Such characteristics are similar to certain p53 gain-of-function missense mutants.

We also found that reprogramming of cells toward acquisition of mesenchymal-like features, induced by either expressing p53Ψ or p53 with gain-of-function mutations, is contingent upon increased production of reactive oxygen species (ROS) by virtue of interaction with cyclophilin D (CypD), a mitochondrial matrix peptidyl-prolyl isomerase known to modulate opening of the mitochondrial permeability transition pore (mPTP). Hence, we propose that p53Ψ encodes a "separation-of-function" isoform that lacks canonical p53 tumor suppressor/transcriptional activities, but is capable of reprogramming cells toward acquisition of mesenchymal-like features in a transcriptionally independent manner. The remarkably similar activities of p53Ψ and certain p53 mutants also suggest that the latter "highjack" a regulated and reversible program (i.e., p53Ψ alternative splicing) that contributes to the biology of p53 mutations during tumorigenesis. Thus, in principle, this implies a possible physiological origin for certain p53 mutations.

Debjani Pal

PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF SIGNAL TRANSDUCTION

N.K. Tonks S. Akshinthala, G. Bencze, F. Chaudhary, G. Fang, N. Krishnan, L. Li, M. Ramesh, I. Rus, M. Yang, S. Zhang

An important aspect of the work of the Tonks lab is to devise creative new approaches to exploiting the protein tyrosine phosphatase (PTP) family of enzymes as targets for therapeutic intervention in major human diseases, including diabetes, obesity, and cancer. At the core of this effort is the study of PTP1B, the prototypic protein tyrosine phosphatase, which Nick Tonks first discovered 25 years ago. It is now established that PTP1B not only plays a role in attenuating insulin signaling, but also plays a *positive* role in promoting signaling events associated with breast tumorigenesis. This suggests that inhibition of PTP1B function may represent a novel therapeutic strategy to address not only diabetes and obesity, but also mammary tumorigenesis and malignancy.

The reversible addition and removal of phosphate to proteins, which is termed protein phosphorylation, is the central feature of the mechanism of signal transduction—the process by which cells respond to stimuli in their environment. The activities of the enzymes that mediate the addition (kinases) and removal (phosphatases) of phosphate groups are coordinated in signal transduction pathways to mediate the cellular response to environmental stimuli, and the function of these enzymes is frequently disrupted in human diseases, including diabetes and cancer. The ability to modulate such signal transduction pathways selectively with drugs holds enormous therapeutic potential. Drugs that target the protein kinases represent breakthroughs in cancer therapy. For example, HER2 is a member of the family of kinases and is amplified and/or overexpressed in several cancers, in particular in ~25% of breast cancer, where it is associated with poor prognosis. The humanized HER2-directed antibody, Herceptin (trastuzumab), is an example of a "rational cancer therapy" for treatment of HER2-positive metastatic breast tumors. It targets HER2 as a unique marker of the cancer cell. Although Herceptin is used frequently and is being presented as a treatment of choice, the overall success rate is low and patients develop resistance to the therapy. The problem of acquired resistance has become an obstacle to the successful application of kinase-directed therapies in general. Therefore, despite the obvious potential, it is anticipated that new alternative therapies, administered alone or in combination with kinase-directed drugs, will represent the way forward. The challenge is to identify such alternative therapies. Considering the intimate cooperation between kinases and phosphatases in the regulation of signal transduction under normal and pathophysiological conditions, the Tonks lab focuses on the PTPs, which have been garnering attention as potential therapeutic targets, but remain a largely untapped resource for drug development.

In following established approaches to developing small-molecule drugs that bind to the active site of an enzyme, industry has found PTPs to be challenging targets for therapeutic development. Although it was possible to generate potent, specific, and reversible inhibitors of PTP1B, such molecules were highly charged, due to the chemistry underlying PTP-mediated catalysis, and thus of limited drug development potential. Consequently, new approaches are required to exploit this important target effectively and reinvigorate drug discovery efforts. One strategy that would avoid targeting the active site of PTP1B would be to look for allosteric inhibitors, which bind at a site remote from the catalytic center, but induce conformational changes in the enzyme that result in inhibition. The Tonks lab has characterized a small-molecule natural product, TRODUSQUEMINE, as such an allosteric inhibitor of PTP1B. This is a unique mechanism of inhibition that will have been missed in the efforts to target PTP1B that have been conducted to date in industry. They have demonstrated that this inhibitor antagonizes the function of HER2 in cell and animal models of breast cancer. In particular, it essentially abrogates metastasis of HER2-positive tumor cells in the NDL2 mouse model of breast cancer.

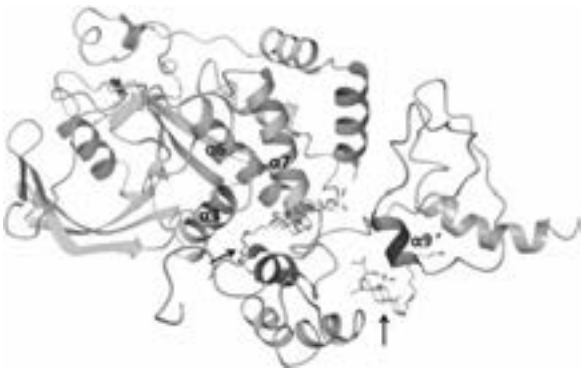

Figure 1. Structure model for PTP1B (residues 1–405) with two molecules of the allosteric inhibitor MSI-1436 (marked with arrows) docked to the two putative binding sites. Important structural features within PTP1B are highlighted.

It is important also to note that TRODUSQUEMINE (also known as MSI-1436) has already been tested in a Phase 1 study that involved 88 obese patients and was found to be extremely well tolerated. Of particular significance is the fact that the effects observed in mice by the Tonks lab were achieved at ~20% of the maximum dose that has been administered to patients. During the last year, discussions have been initiated with the FDA, and a collaboration between the Tonks lab and the Monter Cancer Center at North Shore LIJ has been set up to take TRODUSQUEMINE/MSI-1436 into clinical trials for HER2-positive cancer. In an initial meeting with the FDA, it was indicated that a Phase 1 study of safety and tolerability of TRODUSQUEMINE/MSI-1436 as a single agent in metastatic breast cancer patients would have to be performed. The preparations for this clinical trial are now under way and will represent a focus of effort in 2014.

Although TRODUSQUEMINE/MSI-1436 demonstrates efficacy in an injectable format, it also has limited oral bioavailability. Industry has set oral bioavailability as a hurdle for the next generation of therapies for diabetes. During the last year, the Tonks lab has identified a derivative of TRODUSQUEMINE/MSI-1436 for which they have demonstrated oral bioavailability in a weight loss study in a high-fat diet-induced mouse model of obesity. At this time, they are defining the mechanism of action of this inhibitor. Furthermore, they have identified novel chemical entities that display properties similar to those of TRODUSQUEMINE/MSI-1436, which are currently being validated as inhibitors of PTP1B to identify further new drug candidates.

The Tonks lab is also trying to harness a physiological mechanism for regulation of PTP1B function as a distinct approach to development of therapeutics, particularly in the context of diabetes and obesity. PTP1B is a major regulator of the signaling pathways initiated by insulin and leptin, which controls appetite. Gene-targeting studies demonstrated that PTP1B-null mice are healthy, display enhanced insulin sensitivity, do not develop type-2 diabetes, and are resistant to obesity when fed with a high-fat diet. Furthermore, depletion of PTP1B expression with antisense oligonucleotides elicits anti-diabetic and anti-obesity effects in rodents, as well as human subjects. The Tonks lab discovered that the activity of PTP1B is attenuated by reversible oxidation of an essential cysteinyl residue at the active site of the enzyme. The architecture of the PTP-active site is such that this essential cysteinyl residue displays unique properties that favor its role in catalysis but also render it prone to oxidation. Insulin stimulation of mammalian cells leads to enhanced production of intracellular H_2O_2, which causes reversible oxidation of PTP1B and inhibition of its enzymatic activity, which in turn concomitantly promotes the signaling response to insulin. They have shown that mild oxidation of PTP1B, such as occurs in response to insulin, results in profound conformational changes in the active site of the enzyme that transiently inhibit substrate binding and catalysis. These structural changes, however, are reversible and the enzyme can be reduced back to its active state. Therefore, reversible oxidation of PTP1B in response to insulin provides a mechanism for fine-tuning the signaling response to the hormone. They hypothesized that a conformation-sensor antibody that recognizes the reversibly oxidized form of PTP1B selectively may stabilize the inactive state, inhibit its reactivation by reducing agents, and thereby inhibit phosphatase activity. Using antibody phage display, they generated such conformation-sensor antibodies and demonstrated that expression of these antibodies in cells enhanced insulin-induced signal transduction. Their data provide proof-of-concept that stabilization of the oxidized, inactive form of PTP1B with appropriate therapeutic molecules may offer a novel paradigm for phosphatase drug development. This has the additional advantage that, if one assumes that in responding to insulin the cell targets for oxidation the pool of PTP1B that is important for physiological regulation of the signaling response, then this strategy

will target that pool specifically, possibly also reducing complications of side effects that may accompany inhibition of the native enzyme as a whole.

The antibodies themselves are unlikely to be of use as therapeutics; however, the Tonks lab has now developed an assay that will permit screening of a small-molecule library on an industrial scale to identify drug-like molecules that mimic the effects of the antibodies. A pilot-scale screen has already been completed, demonstrating the feasibility of this strategy and identifying candidate small molecules that stabilize specifically the oxidized form of PTP1B. During the last year, this approach has led to the initiation of a collaboration with a large pharmaceutical company. Studies are under way to test the potential for conformation-sensor antibodies that recognize selectively the oxidized form of PTP1B to promote the signaling response to leptin. If these antibodies are shown also to potentiate leptin signaling, it is anticipated that a full-scale screen of the company's small-molecule collection will be initiated to exploit fully this unique approach to development of PTP inhibitors, thus opening a new strategy for therapeutic intervention in a major disease.

Efforts in the Tonks lab to define PTP function, and wherever possible establish links to human disease, are not restricted to PTP1B. Examples of progress in additional areas include the following.

Characterization of PTP Function in the Control of Migration and Invasion of Mammary Epithelial Cells

The Tonks lab has characterized missing in metastasis (MIM), which is a scaffold protein that is downregulated in multiple metastatic cancer cell lines compared to nonmetastatic counterparts. MIM regulates cytoskeletal dynamics and actin polymerization and has been implicated in the control of cell motility and invasion. MIM has also been shown to bind to a receptor PTP (PTPδ), an interaction that may provide a link between tyrosine phosphorylation-dependent signaling and metastasis. They used small hairpin RNA (shRNA)-mediated gene silencing to investigate the consequences of loss of MIM for the migration and invasion of the MCF10A mammary epithelial cell model of breast cancer. They observed that suppression of MIM by RNA interference (RNAi) enhanced migration and invasion of MCF10A cells, effects that were mediated by enhancing the stability and quantity of PTPδ. Furthermore, analysis of human clinical data indicated that PTPδ was elevated in breast cancer samples when compared to normal tissue. They demonstrated that SRC is a direct substrate of PTPδ, and, upon suppression of MIM, they observed changes in the phosphorylation status of the SRC protein tyrosine kinase: The inhibitory site (Y527) was hypophosphorylated, whereas the activating autophosphorylation site (Y416) was hyperphosphorylated. Thus, the absence of MIM led to PTPδ-mediated activation of SRC. Finally, the SRC inhibitor SU6656 counteracted the effects of MIM suppression on cell motility and invasion. This demonstration of PTPδ-dependent activation of SRC in cells depleted of MIM suggests a new therapeutic strategy for targeting metastasis.

Investigation of the Role of Thioredoxin in Reversible PTP Oxidation

Despite great progress in defining the mechanism of reactive oxygen species (ROS) production, the controls over the levels of ROS in cells, and the scope of potential targets of this modification, the mechanisms underlying reduction and reactivation of the oxidized PTPs have remained relatively underexplored. It is essential that PTPs recover their active form when ROS concentrations decrease so as to limit the response to growth factors/hormones and prevent uncontrolled stimulation of signaling pathways. For the PTPs, redox-active enzymes and small molecules, such as thioredoxin (TRX), glutaredoxin, or glutathione, have been implicated in reduction and reactivation. In a collaborative study with Arne Östman's lab, they demonstrated that TRX was important for reduction and reactivation of PTP1B in the context of PDGF signaling. In a separate study, they used a combination of biochemical analysis, mechanism-based trapping and RNAi-induced suppression to demonstrate the importance of TRX1 for the reduction and reactivation of PTP1B and PTEN. Although TRX is best known as a disulfide reductase, they demonstrated that it has an important role in the reduction and reactivation of the oxidized form of PTP1B, which features a cyclic sulphenamide modification of the active-site cysteine residue. Furthermore, they have shown that these effects of TRX have the potential to fine-tune the signaling

response to insulin. Finally, in addition to highlighting the important role of TRX in reduction and reactivation of PTPs, the TRX trapping mutants illustrate another approach to defining the importance of reversible oxidation in the regulation of PTP function in general and tyrosine phosphorylation-dependent signaling in a broad array of signaling contexts.

Identification of the Anti-Inflammatory Compound BAY 11-7082 as a Potent Inhibitor of PTPs

BAY 11-7082 is an anti-inflammatory compound that has been reported to inhibit IκB kinase activity. The compound has an α,β-unsaturated electrophilic center, which confers the property of being a Michael acceptor; this suggests that it may react with nucleophilic cysteine-containing proteins, such as PTPs. In this study, the Tonks lab demonstrated that BAY 11-7082 was a potent, irreversible inhibitor of PTPs. Using mass spectrometry, they demonstrated that BAY 11-7082 inactivated PTPs by forming a covalent adduct with the active-site cysteine. Administration of the compound caused an increase in protein tyrosine phosphorylation in RAW 264 macrophages, similar to the effects of the generic PTP inhibitor sodium orthovanadate. These data illustrate that BAY 11-7082 is an effective pan-PTP inhibitor with cell permeability, revealing its potential as a new probe for chemical biology approaches to the study of PTP function. Furthermore, the data suggest that inhibition of PTP function may contribute to the many biological effects of BAY 11-7082 that have been reported to date.

Investigation of the Regulation and Function of Receptor PTPα in Breast Cancer Models

HER2/ERBB2 is a member of the epidermal growth factor (EGF) receptor family of protein tyrosine kinases (PTKs) and is amplified and/or overexpressed in ~25% of breast cancer and associated with poor prognosis. Using MCF10A mammary epithelial cells that ectopically express an "activatable" ErbB2 chimera as a model, they demonstrated that NOX-dependent production of hydrogen peroxide occurs rapidly following ERBB2 activation, leading to reversible oxidation of the transmembrane receptor phosphatase PTPα. This inhibited the activity of PTPα, contributing to increased ERBB2 signaling. Furthermore, the suppression of PTPα by shRNA led to ERBB2-dependent increased cell migration, which was characterized by prolonged interaction of GRB7 with ERBB2, increased association of ERBB2 with a β1 integrin-rich complex, and was dependent on GRB7-SH2 domain interactions. Interestingly, the human *GRB7* gene is commonly co-amplified with *ERBB2* in breast cancer, and GRB7 has been implicated in cell migration. Supporting this concept, the ERBB2-dependent migration in PTPα-knockdown cells was suppressed by a GRB7-SH2 domain inhibitor. They demonstrated that PTPα dephosphorylated FAK specifically on Tyr^{407}, and FAK Tyr^{407} phosphorylation was enhanced in cells following ERBB2 activation when PTPα was suppressed, which contributed to the recruitment of vinculin to FAK. Collectively, these data support a role for PTPα in regulating motility of mammary epithelial cells in response to ERBB2 signaling, consistent with a role for the phosphatase in breast cancer.

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UNDERSTANDING PROSTATE CANCER METASTASIS

L. Trotman M. Chen T. Herzka D. Nowak
H. Cho A. Naguib W. Zheng

We study the mechanisms behind the transition from indolent to lethal metastatic prostate cancer. Our aim is to recreate the lethal disease in fast and faithful mouse models that can be studied and used for preclinical trials that help us develop better therapies. PTEN is a haploinsufficient tumor suppressor: Partial loss or degradation results in growth advantage, and in contrast, we found that complete loss triggers senescence arrest. Studying human prostate cancer genomes showed that loss of PTEN and p53 genes was strongly associated with metastatic disease, suggesting that overcoming the senescence brakes leads to disease progression in some 50% of patients. Classic mouse models of this genotype, however, failed to recapitulate metastasis. We are developing a new approach for modeling prostate cancer in mouse, termed RapidCaP. It allows us to generate loss of Pten and p53 genes without the need for breeding and suggests that widespread metastasis is found when the genes are co-deleted. This approach could serve as the first platform for therapy of endogenous metastatic prostate cancer in a preclinical animal model.

At the same time, we study regulation of the PTEN protein because many patients show reduced protein in spite of intact DNA and RNA function.

Molecular and Genetic Analysis of Prostate Cancer Evolution

H. Cho, D. Nowak, T. Herzka, W. Zheng [in collaboration with J. Hicks and M. Wigler, Cold Spring Harbor Laboratory; B. Robinson, Weill Cornell Medical College, New York]

Understanding the progression from indolent to lethal prostate cancer remains the major challenge in the field. Hyperactivation of the phosphoinositol-3 kinase (PI3K)/AKT pathway is common in many cancer types. Our work demonstrated that loss of the capacity to mount a senescence response is critical for the lethal progression event. To define the molecular changes that occur in this transition, we have recapitulated the pre- and postsenescence genetics in vitro using primary cells and analyzed the signatures that are an immediate consequence of breaking the senescence response by loss of p53. Comprehensive analyses of changes in transcription, proteome, and secretome have led to identification of signature responses, which are being validated in human prostate cancer specimens (in collaboration with Dr. Robinson).

In a collaboration with scientists at McGill University, Montreal, we found that amplification of an androgen receptor (AR) target locus is associated with amplification of AR itself, suggesting that enhanced transcription at these sites may promote amplification. Because the region has previously been implicated as an inherited locus for prostate cancer risk, it may emerge as a critical region for genome analysis and prediction of a patient's disease progression.

To validate alterations and key genetic events that are found in the human samples, we are developing more flexible mouse modeling techniques. RNA-interference (RNAi) has been shown to recapitulate key features of gene knockout models, especially given the fact that the majority of tumor suppressors are found in hemizygous deletions in cancer. RNAi-based models of Pten/p53 loss have been generated and followed up for disease initiation and progression characteristics. Most notably, our approach of direct viral injection into prostate has shown highly efficient gene transduction of cancer genes, cre-recombinase, as well as reporter genes, resulting in highly penetrant metastasis. Thus, our approach introduces a fast and flexible method for the generation of prostate cancer and its metastasis that can be tracked in live animals.

Living with Lethal Genes

M. Chen, A. Naguib, T. Herzka, W. Zheng

PTEN is the major negative regulator of PI3K signaling with cell-specific functions that go beyond tumor suppression. In fact, it is surprising that a gene that prevents cells from growing and can cause them to die is constitutively expressed in most healthy tissues. This

raises the fundamental question of how the *PTEN* gene is suppressed to allow for normal growth, tissue repair, and development. We are interested in this question because many cancer types reveal degradation of PTEN protein while the gene remains intact. Our hypothesis is that such cancers indirectly target PTEN by loss of genes that are essential for PTEN maintenance. Thus, understanding the normal processes behind PTEN regulation may unearth critical cancer genes.

Ischemic injury results from insufficient blood flow to organs or tissues, examples of which include stroke and myocardial infarction. PTEN, under normal physiological conditions, acts to antagonize PI3K/AKT-mediated signaling and thereby promotes growth arrest and can trigger apoptosis. However, subsequent to ischemic injury, limiting the ability of wounded tissue to renew or promoting cell death would be detrimental to the healing effort. To our surprise, we learned that nuclear seclusion of PTEN is one mechanism to temporarily suppress its function: 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.

These findings highlight the intersection of PTEN nuclear import and degradation, and we are currently testing the role of nuclear import receptors in regulating PTEN stability and transport.

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Tali Herzka

CANCER MEDICINE LABORATORY/CANCER THERAPEUTICS INITIATIVE

D. Tuveson B. Alagesan, T. Ardito, L. Baker, C. Chio, V. Corbo, B. Creighton, B. Delcuze, E. Elyada, D. Engle, M. Feigin, A. Handly-Santana, C. Hwang, M. Ludwig, D. Ohlund, T. Oni, Y. Park, M. Ponz-Sarvise, H. Tiriac, K. Wright, K. Yu

The Tuveson laboratory uses cancer models and human clinical trials to explore fundamental biology questions and to identify new diagnostic and treatment strategies. Our main focus is pancreatic cancer, a lethal malignancy despite intensive study. For example, we have adapted a new method to culture primary human and mouse pancreatic tissue indefinitely as ductal organoids, enabling deep molecular and therapeutic analyses. Second, therapeutic experiments in mouse models have revealed an important role for stromal interactions on influencing therapeutic response. We are initiating our first clinical trial shared between CSHL and Memorial Sloan-Kettering Cancer Center to investigate a drug delivery approach for pancreatic cancer patients. Collectively, our strategy in the preclinical and clinical arena represents the "Cancer Therapeutics Initiative" at CSHL, a resource that should enable many investigators to do similar work.

Cancer Diagnostics

This work was done in collaboration with D. Pappin (Cold Spring Harbor Laboratory), J. Lewis (Memorial Sloan-Kettering Cancer Center), and R. Hynes (Massachusetts Institute of Technology).

The diagnosis of early-stage cancers in most patients is challenging and usually based on a tissue biopsy. Indeed, our failure to detect nascent malignancies when anatomically localized and potentially surgically curable reflects the lethality of many cancers for which insufficient systemic therapies exist. Cervical cancer best exemplifies this principle, where the introduction of gynecological examinations with histological screening led to a marked decrease in the previously main cause of female cancer deaths worldwide. Additionally, the direct visualization of incipient tumors with a variety of radiological and endoscopic modalities also reduces lung and colon cancer mortality, respectively. These recent advances have established standards for preventive medicine in the United States, although most patients who are ultimately diagnosed with lung, bowel, and other malignancies are missed. Investigational approaches include the development of blood-based biomarkers such as circulating nucleic acids, proteins, cancer cells, exosomes, and immune response biomarkers. These exploratory efforts are still under way, and other than biomarkers for uncommon cancers (e.g., b-HCG for choriocarcinoma, AFP for testicular cancer), they have not yet provided an effective approach that can be utilized in a general or selected population of otherwise healthy individuals. The major barriers to the development of cancer biomarkers are the lack of sensitivity (limits of detection) and specificity (due to similarities to nonmalignant diseases). Accordingly, we have initiated a new research program to develop methods for tumor detection in mice by taking advantage of genetic differences between mice and humans, in efforts to find biomarkers of early-stage, pancreatic preneoplasms. These methods include a combination of biochemical and radiological approaches.

Cancer Therapeutics

This work was done in collaboration with K. Yu (Cold Spring Harbor Laboratory/Memorial Sloan-Kettering Cancer Center) and A. Krainer (Cold Spring Harbor Laboratory).

Pancreatic cancer is refractory to conventional and targeted agents. Although the drug resistance of pancreatic ductal adenocarcinoma (PDA) may represent

unique cell-intrinsic drug resistance mechanisms such as cellular differentiation states and drug exporters, we have shown that the stromal-rich PDA tumors lack a functional vasculature-limiting drug delivery, thus contributing to drug resistance. Hedgehog inhibition and stromal digestion with pegylated hyaluronidase (PEG-PH20) both led to increased perfusion and chemotherapy delivery in PDA tumor tissues, and transient increases in mouse survival. These findings became the basis of a clinical trial using the hedgehog inhibitor IPI926 in combination with gemcitabine for patients with metastatic PDAC, but unfortunately, the first randomized trial was negative and the analysis is ongoing. Early-phase clinical trials with gemcitabine in combination with PEG-PH20 are under way, with interim results expected shortly. Interestingly, PEG-PH20 treatment also specifically increased the delivery of high-molecular-weight agents, providing the opportunity to evaluate novel high-molecular-weight therapeutic agents that are otherwise difficult to deliver to PDAC tissues, including antibodies and nucleic acids.

Dr. Ken Yu, working with the Tuveson laboratory as a CSHL clinical fellow and a medical oncologist at MSKCC, has written a proof-of-concept clinical trial to assess whether PEG-PH20 and related stromal disruption approaches will increase the effectiveness of biological therapeutics in pancreatic cancer patients. His trial will be conducted to establish whether PEG-PH20 can increase the delivery of an FDA-approved anti-EGFR (epidermal growth factor receptor) antibody cetuximab in patients scheduled to undergo surgery for resectable pancreatic cancer. The design will be to administer PEG-PH20 2 d before surgery and treat with therapeutic antibody 24 h later, with surgery following 24 h later. Comparisons will be made to patients who do not receive PEG-PH20, and resected tumors will be assessed for the delivery of the antibody and any biochemical alterations by IHC. Cetuximab is the first antibody to be considered because it binds to EGFR, which is expressed by the majority of PDAC cells and can be easily detected through the chimeric murine portion of the antibody. Cetuximab was not shown to alter the effects of gemcitabine treatment in PDAC patients in a recent SWOG trial, potentially because it is not delivered optimally. Positive findings will motivate the assessment of additional agents in more advanced preclinical and clinical trials, including neoadjuvant trials in locally advanced pancreatic cancer patients and mice to improve resectability and survival.

Besides the limitations in drug delivery observed in pancreatic tumors, we recently reported that the matricellular protein connective tissue growth factor (CTGF) promotes drug resistance in mice with PDA (Neesse et al. 2013a). Our analysis has determined that CTGF promotes cell survival in neoplastic cells by up-regulating the survival factor XIAP. This suggests that multiple matricellular proteins may serve such a role in preventing a response to therapies in PDA. Our organoid approach below is currently being used to establish the mechanism of CTGF function.

Development of Pancreatic Ductal Organoids as a Novel In Vitro Cancer Model for Biological Exploration and Medical Applications

This work was done in collaboration with H. Clevers, Hubrecht Institute, The Netherlands.

To accelerate our molecular and therapeutic work, we established a method to indefinitely culture normal, preneoplastic, and frankly malignant murine and human pancreatic ductal cells in semisolid media (Fig. 1). This approach was based on the pioneering work of Dr. Hans Clevers (Hubrecht Institute, The Netherlands), who demonstrated that intestinal villi could be indefinitely propagated in three-dimensional cultures, and he termed these cultures organoids because they formed normal tissues upon transplantation into murine intestines. Our pancreatic ductal organoid structures obtained from normal and malig-

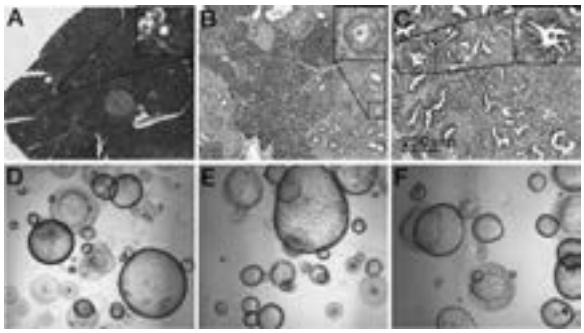

Figure 1. Mouse pancreatic tissues from normal (*A*) PanIN (*B*) and tumors (*C*) were used to isolate the corresponding organoid lines (*D–F*). Scale bar, 200 µm. $n > 3$ samples each.

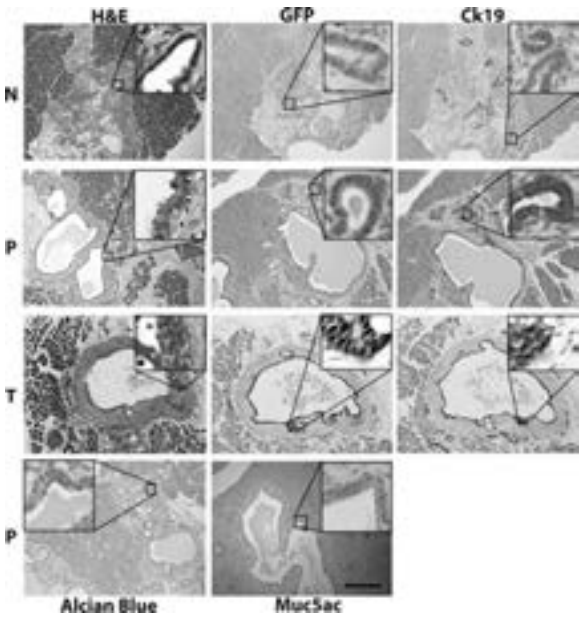

Figure 2. Orthotopic, syngeneic transplantation of GFP-expressing organoids evaluated 1 mo later by H&E and IHC. (*Bottom*) Mucin production (Alcian blue) and Muc5ac expression in transplants from P organoids. Scale bar, 300 µm. $n > 3$ cases each.

nant mouse pancreata resumed their original morphological appearances following orthotopic transplantation, confirming that they represent distinct stages of tumorigenesis (Fig. 2). These cultures will enable the less costly, high-throughput assessment of small molecule and shRNA screens in cell culture, prior to characterization in vivo. Additionally, they can be directly characterized at the genomic, transcriptomic, and proteomic levels to determine the molecular correlates of therapeutic response. This approach will be compared to routine two-dimensional tissue culture systems, and in parallel, they will be compared to the traditional PDX models. If organoids offer advantages to PDX models, we will determine how to extend this to other organ types and how to best utilize this technology for patient benefit. For example, serial organoids from patients under therapy may reveal a model system for interrogating drug resistance and identifying new targets of drug sensitivity, perhaps more robustly than circulating tumor cells (CTCs) as it is challenging to propagate these cells in culture. We also have found that cancer cell lines readily adapt to growth in organoid conditions, suggesting that this approach for CTCs may work.

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RAS AND RHO GTPases AND THE CONTROL OF SIGNAL TRANSDUCTION

L. Van Aelst B. Elmer M. Wang
Y. Tai Y.-T. Yang
C.-L. Wang J.-R. Yu

Research in my laboratory is focused on the role of Ras and Rho GTPase family members in signal transduction. 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 causally linked to both cancer and brain disorders, including mental retardation, schizophrenia, and epilepsy. Our interests lie in understanding how defects in Ras- and Rho-linked proteins contribute to the development of these disease processes. Toward this end, my lab has continued to define the functions of selected GTPases and their regulators and effectors in models of cancer and neurodevelopmental disorders. Below are highlighted selected key projects that have been performed during the past year.

Regulation of Chandelier Cell Cartridge and Bouton Development via DOCK7-Mediated ErbB4 Activation

DOCK7 is a member of the evolutionarily conserved DOCK180-related protein superfamily. The DOCK180 family emerged as a distinct class of Rac and/or Cdc42 GTPase guanine nucleotide exchange factors (GEFs), which have diverse cell-type-specific functions. We initially identified DOCK7 as a novel activator of Rac that is highly expressed in the developing brain. Significantly, sequence variations in DOCK7 have been reported in schizophrenia patients; however, the role(s) of DOCK7 in neuronal development and/or function has remained largely elusive.

We previously reported that DOCK7 has a critical role in the polarization and genesis of newborn pyramidal neurons and that it does so by promoting Rac activity and antagonizing TACC3 (transforming acidic coiled-coil-containing protein 3) function, respectively. Interestingly, in more recent studies examining the expression patterns of members of the DOCK180 family in GABAergic interneurons, we intriguingly observed the presence of DOCK7, among other parvalbumin (PV)-expressing interneurons, in chandelier cells (ChCs) of adolescent/adult mouse brains. ChCs, typified by their unique axonal morphology, are the most distinct interneurons present in cortical circuits. Via their distinctive axonal terminals, called cartridges, these cells selectively target the axon initial segment of pyramidal cells and control action potential initiation; yet, the mechanisms that govern the characteristic ChC axonal structure have remained elusive. The main obstacles have been a lack of unique biochemical ChC markers and versatile methods to target and manipulate gene expression in these cells. This prompted us to develop a method to manipulate gene expression in ChCs so that DOCK7's role in their development could be assessed. On the basis of recent evidence indicating that progenitors in the ventral medial ganglionic eminence (vMGE) provide a source of ChCs, we reasoned it should be possible to target gene expression in nascent ChCs by means of in utero electroporation directed toward the vMGE and found that this is indeed the case. Using this technique, we demonstrated a critical role for DOCK7 in ChC cartridge/bouton development. In particular, knockdown of DOCK7 caused a disorganization of ChC cartridges and a decrease in the density and size of ChC boutons. DOCK7 overexpression elicited essentially the opposite phenotypes. An organized network of ChC cartridges was formed, and both the density and size of boutons were increased. We further found that DOCK7 functions as a cytoplasmic activator of the schizophrenia-associated ErbB4 receptor tyrosine kinase, and importantly that DOCK7 modulates ErbB4 activity to control ChC cartridge/bouton development. Indeed, an ErbB4 mutant with enhanced kinase activity was able to overcome the phenotypes associated with DOCK7 knockdown, whereas silencing of ErbB4 prevented the phenotypes

elicited by DOCK7 overexpression. Thus, our findings define DOCK7 and ErbB4 as key components of a pathway that controls the morphological differentiation of ChCs, with implications for the pathogenesis of schizophrenia.

Multifunctional Role of the X-Linked Mental Retardation Protein OPHN1 at the Hippocampal CA1 Synapse

Oligophrenin-1 (*OPHN1*), encoding a Rho-GTPase-activating protein, was the first identified Rho-linked mental retardation (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 revealed the presence of *OPHN1* mutations in families with MR associated with cerebellar hypoplasia, lateral ventricle enlargement, and/or epilepsy. Most of the *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 both at the pre- and postsynaptic site of the hippocampal CA3-CA1 synapse. We previously reported 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. Interestingly, we more recently found that postsynaptic OPHN1 also has a critical role in mediating a form of plasticity (mGluR-LTD) 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-isoxazole propionic 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).

We further demonstrated that OPHN1's role in mediating mGluR-LTD is dissociable from its role in basal synaptic transmission. mGluR-LTD and the associated long-term decreases in surface AMPARs are dependent on OPHN1's interaction with endophilin A2/3, whereas regulation of basal synaptic strength requires OPHN1's Rho-GAP activity and association with Homer 1b/c proteins. As to how OPHN1 could mediate the strengthening of synapses via interactions with Homer 1b/c and RhoA, we previously showed that OPHN1 becomes enriched in spines in response to synaptic activity and NMDAR (*N*-methyl-D-aspartate) activation, where by locally modulating RhoA/Rho kinase activities (i.e., in the proximity of AMPARs), it contributes to the stabilization of AMPARs. Homer 1b/c proteins, on the other hand, were shown to participate in the positioning of the endocytic zone (EZ) near the postsynaptic density (PSD). The close juxtaposition of the EZ and the PSD enables localized endocytosis and recycling of AMPARs, thereby maintaining a mobile pool of surface AMPARs required for synaptic potentiation. Interestingly, we recently found that a physical interaction between OPHN1 and Homer 1b/c is crucial for the positioning of the EZ adjacent to the PSD, and we obtained evidence that this interaction is important for OPHN1's role in controlling activity-dependent strengthening of excitatory synapses. Disruption of the OPHN1-Homer 1b/c interaction caused a displacement of EZs from the PSD, along with impaired AMPAR recycling and reduced AMPAR accumulation at synapses, under both basal conditions and conditions that can induce synaptic potentiation. Thus, these data indicate that OPHN1 exerts its effects on synapse strengthening by serving at least two distinct roles. Via its interaction with Homer 1b/c, OPHN1 facilitates the recycling and thereby maintenance of a mobile pool of surface AMPARs, whereas via its Rho-GAP activity, OPHN1 contributes to the stabilization of synaptic AMPARs.

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

DOK2 Inhibits EGFR-Mutated Lung Adenosarcoma

Members of the Dok family of adaptor proteins have emerged as key modulators of protein tyrosine kinase (PTK) signaling. Dok1, also known as $p62^{dok}$, is the prototypical family member. It was first identified as a substrate of oncogenic tyrosine kinases, $p210^{bcr\text{-}abl}$ and v-Abl, and found to be a substrate of many endogenous PTKs; hence, it was termed Dok, for downstream from kinases. Since the identification of Dok1, six additional Dok family members have been identified: Dok1 to Dok7. Among them, Dok1 and Dok2 share the ability to bind to Ras-GAP, a negative regulator of Ras. We reported previously that Dok1 attenuates growth-factor-induced cell proliferation and that Dok1 as well as Dok2 possess tumor suppressive activity in the context of myeloid leukemia. Indeed, in collaboration with Dr. Pandolfi's group, we found that mice lacking both *Dok1* and *Dok2* spontaneously develop a CML-like myeloproliferative disease. Significantly, Dok family members do not act only as "tumor suppressors" in the hematopoietic compartment, but also in solid tissues, such as the lung. Single, double, or triple compound loss of *Dok1*, *Dok2*, and *Dok3* in mice results in lung adenocarcinoma with penetrance and latency dependent on the number of lost Dok family members.

In more recent collaborative studies with the Pandolfi lab, we examined in more depth the role of Dok2 in suppressing EGFR-driven lung tumorigenesis, especially since *DOK2* was identified as a candidate human lung tumor suppressor gene. Of note, somatic mutations in the EGFR proto-oncogene occur in ~15% of human lung adenocarcinomas, and the importance of EGFR mutations for the initiation and maintenance of lung cancer is well established from mouse models and cancer therapy trials in human lung cancer patients. Interestingly, we found that genomic loss of *DOK2* is associated with EGFR mutations in human lung adenocarcinoma. These findings led us to postulate that loss of *DOK2* could combine with EGFR mutations to promote lung tumorigenesis. We tested this hypothesis using genetically engineered mouse models and found that loss of Dok2 in mice accelerates lung tumorigenesis initiated by oncogenic EGFR, but not by mutated Kras. Moreover, we found that DOK2 participates in a negative feedback loop that opposes mutated EGFR; EGFR mutation leads to recruitment of DOK2 to the EGFR and DOK2-mediated inhibition of downstream activation of RAS. Thus, these data identify DOK2 as a tumor suppressor in EGFR-mutant lung adenocarcinoma.

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REGULATION OF MALIGNANT GLIOMA HETEROGENEITY AND LINEAGE DEVELOPMENT

H. Zheng B. Guo L. Zhang
L. Klingler

Malignant glioma is the most common and lethal type of brain tumor in adult patients. The current standard care for malignant glioma includes maximal surgical resection, followed by radiation with adjuvant chemotherapy for the residual infiltrative tumor component. Despite these aggressive treatment efforts, the disease invariably returns. In its most aggressive form, glioblastoma (GBM) has a median survival of only 12–15 mo with a 5-yr survival rate of <5% after initial diagnosis. Unfortunately, refinements of available therapeutic modalities including microneurosurgery, radiation, and chemotherapy in the last several decades have not substantially improved patient survival. The long-term goal of our research is to define the complex biology of malignant glioma pathogenesis with the aim of translating the developed knowledge into patient benefits. Along this line, the research in our group has been focused on two major areas: (1) developing various genetically engineered animal models to recapitulate the process of human glioma pathogenesis and using these animal models as tools to investigate in vivo tumor initiation, progression, and their response to various therapeutic treatments and (2) applying an integrated approach combining model systems, neural stem cell biology, and RNA interference (RNAi) to identify molecular and developmental programs relevant to glioma pathogenesis and treatment. Particularly, we are interested in genetic and epigenetic pathways involved in controlling neural precursor cell fate determination. We believe that an improved understanding of the developmental programs governing the self-renewal and differentiation processes along the neural progenitor–glial axis and, by extension, the glioma stem cells–progeny axis, will serve instrumentally in guiding future development of efficient treatments targeting this dreadful disease.

Characterizing EGFR-Targeted Therapeutic Resistance

The epidermal growth factor receptor (EGFR) is a transmembrane protein that belongs to a family of receptor tyrosine kinases. In human malignant glioma, amplification and mutation of *EGFR* represent the signature genetic abnormalities encountered in ~40%–50% of patients, a fact that makes EGFR a compelling candidate for targeting therapy. A range of potential target therapies, particularly small molecular inhibitors of its tyrosine kinase activity, are currently in development or in clinical trials for the treatment of malignant gliomas. Despite their notable therapeutic impact for treating other types of cancers, efficacy of these clinically approved small-molecule EGFR inhibitors in malignant glioma trials has so far been limited by both upfront and acquired drug resistance, and any responses have been unrelated to *EGFR* amplification status. These clinical observations question whether EGFR is a viable therapeutic target for malignant gliomas. There are three potential causes of the clinically observed EGFR inhibitor resistance in glioma: (1) EGFR might not be essential for glioma cell survival, and therefore the EGFR cannot be targeted for glioma treatment, (2) the drugs may not penetrate the blood brain barrier, a general concern for drugs targeting central nervous system diseases, and (3) the brain-cancer-specific EGFR and mutant may not be particularly sensitive to the current small-molecular EGFR kinase inhibitor. To address these questions, we developed a malignant glioma animal model driven by the tetracycline-induced overexpression of a malignant glioma-specific EGFR mutant with concurrent brain-specific inactivation of *Ink4a/Arf* and *Pten* tumor suppressors. The tightly tetracycline-controlled mutant EGFR expression in this case ensures a faithful model system to mirror clinically EGFR targeting therapy and provides us with an opportunity to dissect the resistance mechanism(s) in vivo. By using both genetic and pharmacologic approaches, we demonstrated that genetic ablation of mutant EGFR protein expression leads to acute glioma regression, confirming that EGFR and its mutants are indeed therapeutic targets for

malignant glioma treatment. Importantly, the acute response caused by genetically suppressing EGFR protein expression is in stark contrast to the poor outcome from EGFR kinase inhibitor treatment, which only marginally decelerated tumor growth despite the displayed potency of inhibiting EGFR phosphorylation. Our findings strongly suggest that EGFR kinase activity is not an absolute indicator of overall EGFR oncogenic functions in malignant gliomas as had been thought. Therefore, we believe the next generation of therapies will need to target both EGFR kinase-dependent and -independent activities in order to achieve optimal efficacy against malignant gliomas carrying *EGFR* amplification and/or mutations. We currently are focusing on deciphering the EGFR kinase-independent activities and analyzing their contribution to glioma progression and drug resistance.

Epigenetic Regulation during Glioma Pathogenesis

Epigenetic regulation is developmentally defined as heritable changes in cellular and organismal phenotypes without altering the underlying genetic information and is best exemplified by cell fate determination. During development, epigenetic programs work in concert with genetic mechanisms to regulate transcription and control cell fate determination. As one key event in glioma pathogenesis is corruption of cell lineage differentiation programs, we reason that glioma cells, particularly the G-TICs, are dependent on distinct epigenetic networks to sustain their aberrant differentiation state and therefore are sensitive to perturbations of certain epigenetic structures. In collaboration with Scott Lowe and Chris Vakoc's groups here at CSHL, we previously had initiated a project to probe the epigenetic networks essential for maintaining glioma cell differentiation state. In a comprehensive epigenetic screen of a customized small hairpin RNA (shRNA) library targeting epigenetic regulators, we had identified multiple chromatin modulators including bromodomain containing 4 (Brd4) that are important for glioma cell self-renewal. Starting from this small panel of chromatin modulators, we used an assortment of genetic and pharmacological assays to narrow down candidates to those whose suppression sensitizes glioma cells to differentiation. Interestingly, we found that not all glioma cells respond the same to individual candidate suppression. For example, although Brd4 inhibition did in general slow down glioma cell growth, we noticed that different lineages of glioma cell lines present very different sensitivities to the Brd4 inhibitor. In particular, we observed that the glioma cells with high neuronal lineage marker expression were generally more sensitive to Brd4 inhibition. Consistently, Brd4 inhibition preferentially eliminates neuronal lineage cells during differentiation of cultured neural stem cells. These findings indicate that different subtype of gliomas may depend on distinctive sets of chromatin modulators to maintain their propagation. In addition, identification of the unique dependencies with different subtypes of gliomas will likely provide insight into mechanistic actions of these specific candidates, possibly forming a basis for future patient stratification.

In another effort to probe chromatin modulators for tumor suppressor genes whose suppression disrupts neural progenitor cell differentiation, we have identified multiple interesting candidates, including alpha thalassemia/mental retardation syndrome X-linked (*ATRX*). ATRX is a Rad54-like ATP-driven DNA translocase belonging to the SWI/SNF family of chromatin remodulators, and its germline mutation causes a form of syndromal mental retardation with multiple developmental abnormalities. Recently, frequent *ATRX* somatic frameshift and nonsense mutations that completely abolish protein function have been identified in human pediatric and adult gliomas, pancreatic neuroendocrine tumors (PanNETs), as well as other cancers of the central nervous system (CNS), suggesting a tumor suppressor role of ATRX. But despite some evidence suggesting that Atrx might be involved in facilitating a replication-independent histone variant incorporation into chromatin, in large part, its molecular functions remain unclear. In our current studies, we found that suppression of Atrx expression can significantly promote glioma initiation from murine NSC cells deleted of p53 and Pten tumor suppressor genes. Functionally, we demonstrated that Atrx has an important role modulating neuronal differentiation, although not their lineage determination. By using chromatin immunoprecipitation and sequencing technologies, we further established the genome-wide distribution of Atrx protein in mouse neural stem cells. Our data reveal that Atrx

protein targets tandem repeat sequences and likely regulates expression of the genes associated with these tandem repeats during the process of neural stem cell lineage differentiation. Currently, we are characterizing Atrx molecular targets responsible for its roles in neural stem cell lineage differentiation and glioma pathogenesis.

PUBLICATIONS

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Stephan Klingler

NEUROSCIENCE

How does the brain encode stimuli from the outside world, within and across sensory modalities, to generate specific perceptions that 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 the 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 are engaged 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 surroundings, despite apparent variations in their features. Furthermore, animals aptly extract relevant information about environment across different sensory modalities, combining olfactory, visual, or auditory cues. By recording neuronal activity in the input and output layers of the olfactory bulb, as well as feedback from olfactory cortical areas and neuromodulatory signals, Albeanu and his team aim to understand computations 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 there are many more information output channels leaving the olfactory bulb for the cortex than there are inputs received from the nose. They are currently investigating how this diversity of bulb outputs is generated, as well as how downstream areas, such as the piriform and parietal cortex, make use of such information during behaviors.

The study of decision-making provides a window into the family of brain functions that constitute cognition. It 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. Lab members use carefully designed paradigms that encourage experimental subjects to deliberate over incoming sensory evidence before making a decision. Recent results show that rats and humans have a statistically similar decision-making ability. To connect this behavior to its underlying neural circuitry, the researchers 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.

Research in **Josh Dubnau**'s lab is concentrated on two different questions. First, Dubnau and his team are investigating mechanisms of memory using *Drosophila* as a model system. A second area of research is focused on uncovering mechanisms of neurodegeneration that underlie amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). Work in the Dubnau lab has suggested a novel hypothesis to explain neurodegeneration in these disorders. They discovered that awakening retrotransposons in the genome of some brain cells might be responsible for

causing cell death. Retrotransposons are virus-like repetitive elements that are encoded in the genome and are capable of replicating and inserting into new chromosomal positions. This can lead to DNA damage and cell death by a process known as apoptosis. The lab is investigating this hypothesis for ALS/FTLD using a multidisciplinary approach that includes experimental work with fly, mouse, cell culture, and human postmortem tissue. Computational analyses of genomic data are performed in collaboration with Molly Hammell's group. If the retrotransposon hypothesis is correct, it will change the trajectory of neurodegeneration research and have obvious clinical impact. Retrotransposon RNAs and proteins are promising new diagnostic markers and potentially important therapeutic targets.

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, to identify signaling pathways that control generation of new neurons, and to search for neuronal and neuroendocrine circuits involved in mood regulation. Recent experiments suggest 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. It also explains why multiple brain trauma and prolonged neurodegenerative disease may lead to accelerated decrease of cognitive abilities. In other research, the team has identified stem cell targets of various therapies used for treating depression and developed a general platform to determine the effect of drugs and therapies and predict their action. The team is now focusing on the signaling landscape of neural stem cells and on their interaction with the surrounding niche. Enikolopov's group is also focusing on other types of stem cells in the organism. Their latest discovery, with a team at Cornell University, relates to a new type of stem cell in the ovary that normally heals the ovarian tissue after an oocyte is released, but easily transforms to become malignant and generate tumors. The team is now using these discoveries to reveal how stem cells relate to neural and oncological disorders.

Hiro Furukawa's lab studies receptor molecules involved in neurotransmission. Its members mainly focus on the 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. The Furukawa lab is working to solve the three-dimensional structure of the very large NMDA receptor by dividing it into several domains. They 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, fluorescence 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. During the last several years, the team discovered and mapped several regulatory sites in specific classes of NMDA receptors, progress that now opens the way to the development of a new potential class of drugs to modulate the receptor activity.

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 they are able to manipulate the function of specific cell types with remarkable precision. Huang is particularly interested in circuits that use GABA, the brain's primary inhibitory

neurotransmitter. The lab's work has direct implications in neurological and psychiatric illness such as autism and schizophrenia, which involve altered development and function of GABAergic circuits. Huang's team also previously 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. This has been used to 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. They 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. The team's most recent observations show that GABA also regulates the process by which synapses are pruned after they have been formed. The lab has also made good progress in studying perturbations in the developing GABAergic system in a mouse model of Rett syndrome, one of the autism spectrum disorders. In a recent landmark study, the team also made a major breakthrough in determining the origin of (and the genetic mechanisms that specify) the cortex's powerful and enigmatic chandelier cells. This is a critical class of inhibitory brain cells, and the team showed that chandelier cells are born in a previously unrecognized portion of the embryonic brain, which they have named the VGZ (ventral germinal zone).

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 its 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. This year, the Kepecs lab was able to link foraging decisions—the choice between staying or going—to a neural circuit and specific cell types in the prefrontal cortex. In other work, they identified a class of inhibitory neurons that specializes in inhibiting other inhibitory neurons in the cerebral cortex and conveys information about rewards and punishment. Through 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 what parameters for the process are set by genetic factors. Even less is known about the assembly of the neural net within the mouse olfactory system, which, in the end, enables the individual to distinguish one smell from another with astonishing specificity and to remember such distinctions over time. These are among the challenges that engage Koulakov and his team.

Dysfunction of synapses in the brain is thought to have an important role in the pathogenesis of major psychiatric disorders, including depression, anxiety, and schizophrenia. But what are the causes? Where in the brain does the dysfunction occur? How does it result in the behavioral symptoms of illness? To address these issues, **Bo Li** and colleagues are studying, in animals, normal synaptic plasticity underlying adaptive behaviors and synaptic aberrations responsible for maladaptive behaviors that are related to depression, anxiety, and schizophrenia. Their long-term goal is to develop methods allowing the manipulation of activity in specific brain circuits in order to change disease-related behaviors. Li's group uses 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 stereotaxic virus injection, RNA interference (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 attention deficit, a cognitive impairment that is consistently observed in schizophrenia. This year, Li and his lab members demonstrated that neurons in a tiny area of the mammalian brain called the central amygdala encode fear memory and control fear expression. These findings laid the foundation for future work aimed at understanding the circuit mechanisms of anxiety disorders, in particular post-traumatic stress disorder (PTSD).

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 colleagues are generating a brain-wide connectivity map for the mouse using a shotgun approach, where neuronal tracer substances are injected systematically on a grid in the brain. Currently, ~500 tracer-injected mouse brains may be viewed through a virtual online digital microscope on the project portal (http://mouse.brainarchitecture.org). The project requires a petabyte of data, posing big-data computational challenges that the lab is finding novel ways of meeting. In another application of whole-brain digital neuroanatomy, Mitra is collaborating with Josh Huang to characterize the distribution of the cell bodies and processes of subtypes of GABAergic neurons in mouse brains, to understand the differences between a normal mouse and mouse models of autism spectrum disorders. In parallel, Mitra is undertaking theoretical work at the interface between physics, engineering, and biology by bringing methods from statistical physics to bear on problems in network control theory and multivariable statistics. Biological networks involve large numbers of variables, and it is expected that insights and analytical methods derived from this work will apply to biological networks such as the whole-brain network being determined in the Mouse Brain Architecture Project.

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 multiple 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. This year, they used this method to describe the first whole-brain activation map representing social behavior in normal mice. They are currently focusing on using this approach to study brain activation changes in two mouse models of autism: the 16p11.2 df/+ mouse model, which shows an increased propensity to seizures and hyperactivity, and the CNTNAP2 knockout mouse model, which shows abnormal social behavior.

Stephen Shea's lab studies the neural circuitry underlying social communication and decisions. He uses natural social communication behavior in mice as a model to understand circuits and processes that are evolutionarily conserved and therefore shared broadly across species, likely contributing to disorders such as autism. 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. 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 (granule cells or GCs) that has long been hypothesized to be crucial for memories, but has resisted direct study. They have developed methods for recording, giving them the first glimpse of the dynamics of these cells while the animal is learning an odor. The results show unexpectedly complex population dynamics among the GCs that were independently predicted by a model of odor learning developed in Alexei Koulakov's lab. The two labs are collaborating to discern how GC population activity gets integrated by olfactory bulb output neurons and to pinpoint the synaptic circuit that underlies this form of learning. In parallel, another member of the lab is using imaging techniques to determine how memories are stored among broad neuronal ensembles, at a different level of the system. Recently, the lab made a key breakthrough, developing the ability to record from GCs in awake animals and discovering that their activity is dramatically modulated by state of consciousness. Finally, the Shea lab completed a series of studies of a different form of social recognition: auditory recognition of pup vocalizations by their mothers. Through this research, they have shown that a mouse model of Rett syndrome exhibits deficits in communication and learning not unlike those in human patients. Grants from the Simons and Whitehall Foundations are allowing the lab to extend this work by directly linking these deficits to the action of the gene *MeCP2* in the auditory cortex.

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, what ion channel properties change? These are the questions that drive research in **Glenn Turner**'s laboratory. His team addresses these questions by tracking neural activity using a combination of different techniques. Using electrophysiological methods, they can examine individual neurons with very high resolution, monitoring synaptic strength and spiking output. They have 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 them to actually predict the accuracy of memory formation in separate behavioral experiments. This year, the Turner lab was able to map the activity of a particular region of the brain that is associated with learning and memory. They found that a remarkably small number of neurons are required for flies to distinguish between odors. The Turner lab also studied the role of a specific type of cells, known as Kenyon cells, that receive input via several large claw-like protrusions. These neurons use their claws to recognize multiple individual chemicals in combination in order to remember a single scent. By examining the effects of learning-related genes on these processes, they can in the future 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. Recently, the lab found that when a rat makes a decision about a sound, the information

needed to make the decision is passed to a particular subset of neurons in the auditory cortex whose axons project to a structure called the striatum. In the second major line of work in the Zador lab, they are developing new methods for determining the complete wiring instructions of the mouse brain at single-neuron resolution, which they term the "Connectome." In contrast to previous methods, which 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 just thousands of dollars.

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 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 also has succeeded in reversing memory deficits in mutant flies, work suggesting longer resting intervals for NS patients might reverse their memory deficits. They also identified a means of reversing memory loss in fruit flies while suppressing brain plaques similar to those implicated in Alzheimer's disease by blocking epidermal growth factor receptor (EGFR) signaling, a pathway commonly targeted in cancer. 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. This year, the Zhong lab explored how neurons control our response to different scents, offering insight into how the brain distinguishes between food odors that are attractive and repulsive. They discovered that neurons expressing a particular peptide were only activated by food odors, and the amount of activation predicted how much a fly was attracted to a particular odor.

UNDERSTANDING NEURONAL CIRCUITS IN THE MAMMALIAN OLFACTORY BULB

F. Albeanu F. Anselmi H.G. Chae P. Alexandre Garcia Da Silva M. Koh G. Otazu Aldana
A. Banerjee M. Davis P. Gupta F. Marbach B. Rebouillat

The focus of our research group is to understand how neuronal circuits encode and interpret inputs from the environment and lead to meaningful behaviors. Toward this end, we use optogenetic methods (such as fast multiphoton laser-scanning imaging of genetically encoded neuronal activity reporters or patterned illumination of 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. behaving, 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, outputs, and feedback loops in different layers of the circuit. We modulate activity of select neuronal populations in a reversible manner in awake animals to understand how fundamental sensory encoding problems are solved by the olfactory bulb (OB) and olfactory (piriform) cortex networks.

Characterizing the Input-Output Transform of the Olfactory Bulb

In the 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 OB 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:* 210 [2009]). From each glomerulus, a few dozen

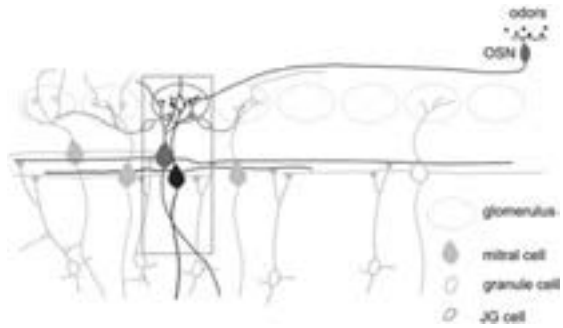

Figure 1. Main olfactory bulb wiring schematics.

mitral cells (principal output neurons of the OB) carry the output further 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 digital micromirror device (DMD)-based patterned photostimulation (Fig. 2), we found that sister cells carry different information to the cortex: Average activity represents shared glomerular input, and phase-specific information refines odor representations and is substantially independent across sister cells (Dhawale et al., *Nat Neurosci 13:* 1404 [2010]).

Activity in the OB 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, GCaMP6) and light-gated switches of neuronal activity (ChR2, Halorhodopsin, Arch) in different bulbar neuronal types. We monitor the

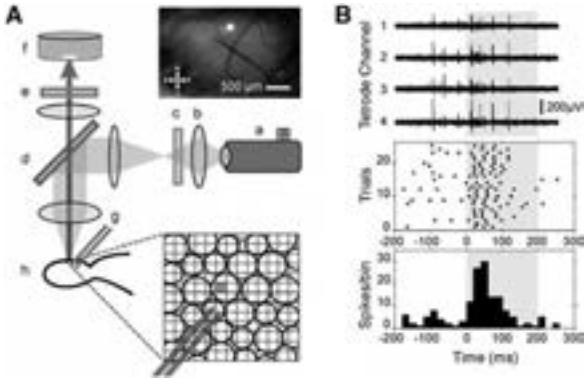

Figure 2. DLP patterned illumination in ORC mice maps the parent glomeruli of mitral cells in vivo. (*A*, *left*) A DLP projector coupled to a system of three lenses delivered light patterns to activate individual glomeruli on the dorsal surface of the bulb. A CCD camera was used to visualize the bulb surface and record the position of the light stimulus. (*Top panel*, *right*) Dorsal surface of the bulb with a tetrode positioned in the mitral cell layer. One square light spot is being projected onto the surface of the bulb. (*Inset*) Cartoon schematic of glomeruli on the bulb, a subglomerular-size light spot and dual tetrodes positioned in the mitral cell layer. (*a*) *A* DLP projector, with color wheel removed from optical path, (*b*) focusing lens, (*c*) blue excitation filter, (*d*) dichroic mirror, (*e*) emission filter, (*f*) CCD camera, (*g*) dual-tetrode, (*h*) olfactory bulb. (*B*, *Top*) Raw voltage traces corresponding to the four channels of a tetrode showing light-induced changes in firing patterns of the units recorded. (*Center*) Raster plot shows changes in spiking activity of a single isolated unit across 25 light stimulation trials, each 200-msec long; dots mark spike occurrence times. (*Bottom*) Peristimulus time histogram (PSTH) with 25-msec time bins, summing spikes over the trials shown in the *center* panel.

inputs (glomerular activity patterns) and the outputs (mitral cell firing) in response to numerous odorants across a wide range of concentrations (five orders of magnitude), as we systematically perturb the activity of the input nodes (glomeruli) and of different 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 we use optical imaging readouts via multiphoton microscopy in vivo to understand what computations the OB performs.

We are currently investigating the roles of two classes of interneurons: short axon cells (SAs) 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.

Long-Range Interactions between SAs and External Tufted Cells Gate the Output of the Glomerular Layer of the OB

Odors elicit distributed activation of input nodes (glomeruli) on the OB. This necessitates long-range interactions among coactive glomeruli. Long-range projecting SA cells provide the earliest opportunity for such cross-talk. SA cells, in the glomerular layer, receive inputs from olfactory sensory neurons (OSNs) and/or external tufted (ET) cells and release both GABA and dopamine, synapsing onto ET cells as far as tens of glomeruli away (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 to odor stimulation by wide-field microscopy across a broad 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 with respect to the OB 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 in mice expressing ChR2 and Halorhodopsin, respectively. In parallel, we developed complementary tools to silence SA cells pharmacogenetically using inhibitory designer receptors exclusively activated by designer drugs (DREADDs) and to monitor mitral cell activity at the population level by retrograde expression of GCaMP3.

Using optogenetic and pharmacogenetic approaches, we find that SA cells influence distant M/T cells by modulating local excitatory interneurons (ET cells). Synaptic SA action enables long-range suppression of ET cells. In addition, we find electrical coupling between SA and ET cells that may facilitate local synchronization of ET-SA responses. Our results indicate that ET cells are the gatekeepers of glomerular output and prime determinants of M/T responsiveness. We

propose that long-range SA-ET action is fundamental for gain control and contrast enhancement by allowing multiple co-active inputs to alter the output of any given glomerulus, before reaching M/T cells.

Characterization of Granule Cell Odor Responses in Awake Head-Fixed Mice

GCs mediate both lateral and recurrent inhibition by forming reciprocal synapses with M/T cells, the principal output neurons of the OB. GCs receive glutamatergic inputs both from MT cells and from feedback axons originating in the olfactory cortex. GCs are the most numerous cells in the OB (~90%), outnumbering the M/T cells by two orders of magnitude, and further represent the main target of direct feedback from the olfactory cortex.

Computational models and behavioral studies have suggested critical roles for these cells in olfactory processing and learning. However, very little is known about their response properties in vivo, because of technical difficulties in electrophysiological recordings from these small-sized neurons.

To begin characterizing the odor response properties of GCs, we used a Cre-LoxP approach to express a genetically encoded calcium indicator (GCaMP6f) in the GCs (CST-Cre) and multiphoton imaging to monitor the odor-evoked responses of GCs (250–350-μm deep) in awake head-fixed mice. GCs showed robust spontaneous activity and were sparsely activated upon odor presentation, displaying a diverse range of enhanced and suppressed, ON, OFF, and ON-OFF responses. Enhanced responses were more common than suppressed responses (65% vs. 35%). Furthermore, a significant fraction (~25%) of GCs exhibited characteristic enhanced OFF responses, independent of stimulus duration. Pairwise analysis of GCs monitored simultaneously indicates that neighboring GCs are as diverse in their odor responses as are pairs of distant cells. No spatial clustering of similarly odor-responding GCs was apparent within a 350-μm range. Enhanced responsive GCs increased monotonically in number across concentrations. Suppressed and OFF-responsive GCs varied in a complex fashion with concentration.

To dissect the contribution of the corticobulbar feedback to GC activity, we are using pharmacological and optogenetic manipulations of the cortical input in tandem with multiphoton imaging of GCs and M/Ts. Furthermore, we are currently monitoring how GC responses evolve during learning and performing of olfactory detection and discrimination tasks.

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), mixtures (A and B), or different concentrations of the same odorant (A) may all result in modulation of odor maps along many (if not all) of these features. It is unknown how the downstream circuitry (M/T cells, olfactory cortex) interprets these variations and assigns meaning to them, in order to segregate odor identity from changes in concentration or components within/from a mixture.

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 OSNs or in a single type of OSN (Olf151). 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 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 under naturalistic conditions to identify odors and their attributes, such as concentration, temporal fluctuations, and 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.

To this end, we are training head-fixed and water-restricted mice to identify a target stimulus ("A") as being unique from other nontarget ("not A") stimuli by licking a "Left" versus "Right" water reward port placed on either side of the mouth (Fig. 3). Animals are reinforced with water reward for correct choices and a mild air puff as punishment upon licking of the wrong port (Fig. 3). We are using wide-field imaging (intrinsic, fluorescence) and two-photon microscopy to monitor the glomerular input maps evoked by the target ("A") and the non-target stimuli ("not A") as mice learn and perform the task. These activity patterns are further used as templates to design photostimulation masks for perturbation of stimulus-induced activity patterns during behavior. Once the task has been learned to >85% accuracy, we reversibly perturb the stimulus "A" activity pattern and determine the behavioral threshold at which an altered activity pattern is classified as "not A."

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 among different neuronal layers. Anatomical studies show massive feedback projections from higher brain areas to the sensory periphery that often outnumber direct sensory inputs. The interplay of feed-forward and feedback signals has been proposed to be fundamental for learning and memory recall. Although rich cortical feedback projections innervate the OB, to date, little is known about their contribution to olfactory processing. Corticobulbar feedback innervates multiple bulb layers, but it primarily targets the granule cells that form extensive dendrodendritic synapses with M/T cells.

Our approach is to understand the role of corticobulbar feedback (Fig. 4) in the identification of an odor, invariant of fluctuations in its concentration and/or timing 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 monitor the activity of feedback inputs and their targets via high-speed scanning multiphoton microscopy and multitetrode recordings.

To date, little is known about the contribution of cortical feedback to olfactory processing in the OB in response to novel or previously encountered odors. To examine how cortical feedback shapes input processing in the OB, we use GCaMP5 and GCaMP6 signals to monitor the odor-evoked responses of feedback fibers in awake head-fixed mice. Feedback fibers showed rich, locally diverse, and brief (<1 sec) spontaneous activity in the majority of imaged boutons. Individual boutons were sparsely activated across odors, resulting in both enhancement and suppression compared to baseline activity. Strikingly, we observed roughly two

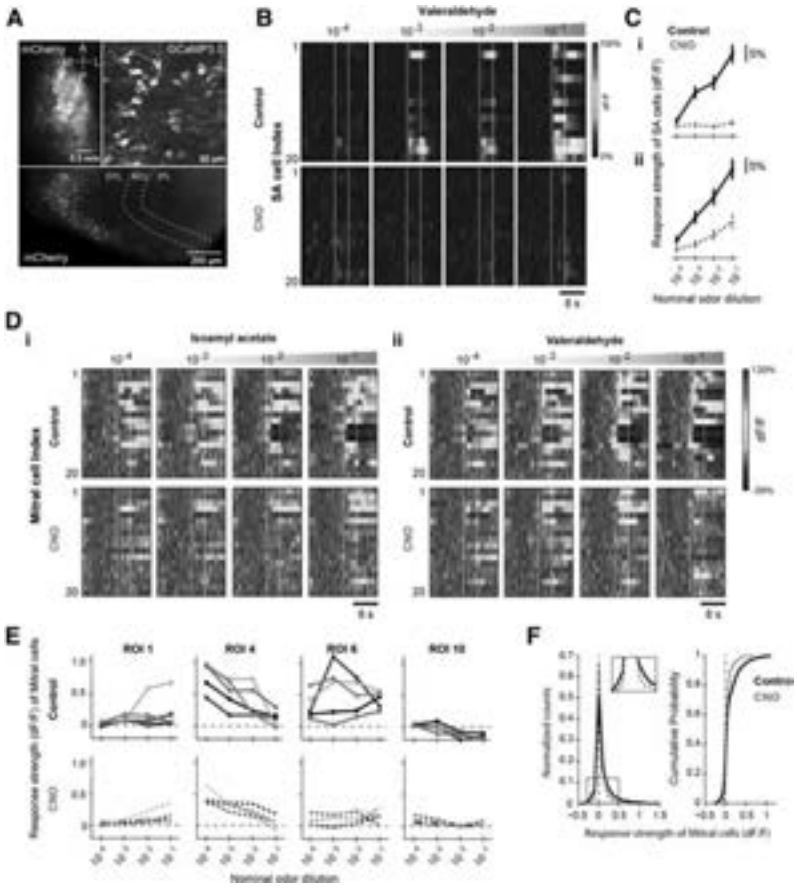

Figure 3. Pharmacogenetic silencing of SA cells suppresses odor-evoked M/T excitation and inhibition. (*A*) Images from the olfactory bulb of a DAT-Cre × Ai38 mouse injected with DIO-DREADDi-mCherry AAV2.9 virus in the glomerular layer and with PRV-Cre in the piriform cortex. (*Top*, *left*) Widefield image showing mCherry expression in the exposed olfactory bulb. (*Top*, *right*) In vivo multiphoton image of resting fluorescence showing GCaMP3.0 expression in M/T cells. (*Bottom*) Confocal image showing selective expression of mCherry expression only in the glomerular layer. (*B*) Baseline-subtracted, normalized GCaMP3.0 signals from an example imaging session of SA cell responses to increasing concentrations of heptanal before (control, *top*) and after CNO (clozapine-*N*-oxide) injection (*bottom*). Stimulus concentrations are reported as nominal dilution in mineral oil. Each row represents an individual SA cell (ROI) in the same field of view. Color indicates normalized change in fluorescence with respect to pre-odor baseline (dF/F). Dotted lines indicate odor presentation (4 sec). Images were acquired at a frame rate of 5 Hz. (*C*, *i*) Average odor-evoked response (dF/F) of all SA cells (20 ROIs) shown in B, across five odors as a function of increasing odor concentration. Dotted lines show responses before and after CNO injection. Error bars indicate standard error of mean. (ii) Average odor-evoked response (dF/F) of all responsive SA cells (110 cells, three mice), across five odors as a function of increasing odor concentration. Dotted lines show responses before and after CNO addition. Error bars indicate standard error of mean. (*D*) Baseline-subtracted, normalized GCaMP3.0 signals from an example imaging session of M/T cells to increasing concentrations of isoamyl acetate (*i*) and valeraldehyde (*ii*) before (control, *top*) and after (CNO, *bottom*) CNO injection. Stimulus concentrations are reported as nominal dilution in mineral oil. Each row represents an individual M/T cell (ROI). Color indicates relative change in fluorescence with respect to pre-odor baseline (dF/F). Dotted lines indicate odor presentation. Images were acquired at a frame rate of 5 Hz. (*E*) Average normalized odor-evoked response (two to three repeats) of four example M/T cells (ROIs) shown in *D*, to five odors, as a function of increasing odor concentration, before (control, solid lines, *top*) and after (CNO, dotted lines, *bottom*) CNO injection. Colors indicate individual odors (same as Fig. 2g). (*F*) Summary histogram (*left*) and cumulative distribution (*right*) of M/T odor responses as a function of normalized response strength (dF/F) before (black) and after (red) CNO addition. 49 cells, 842 cell-odor pairs, three mice.

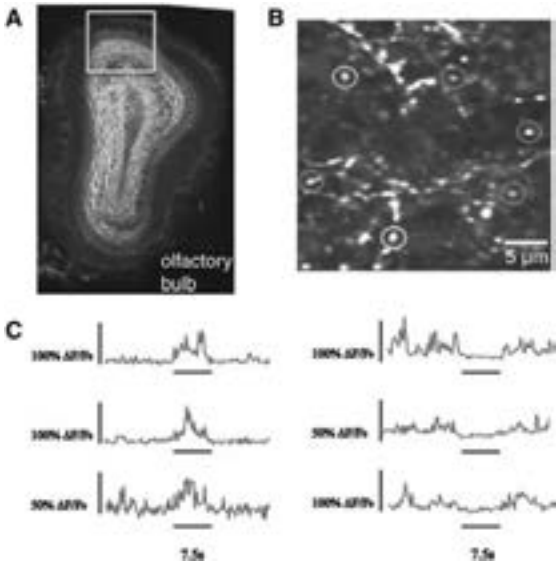

Figure 4. Odor-evoked responses in cortico-bulbar feedback fibers. (*A*) Cortico-bulbar feedback fibers labeled with GCaMP5; (*B*) feedback fiber boutons in an optical plane 230-µm below the surface; (*C*) example responses to three different odors (ethyl tiglate, 2-heptanone, and *p*-anis aldehyde) for six synaptic boutons from the imaged field of view.

types of bouton responses suggestive of distinct piriform cortex output channels. Approximately 40% of the imaged boutons showed purely enhanced responses, whereas ~55% of boutons were consistently suppressed by odors. Only ~5% responded through both enhancement and suppression to different odors. The enhanced and suppressed responses to odors were not just mirror images of each other: Suppressed bouton responses maintained their suppression for several seconds following odor presentation, whereas enhanced bouton responses tracked the temporal dynamics of odors more faithfully. These observations suggest that transient odor input can trigger long-lasting activity (suppression or enhancement) that may further impact bulbar dynamics. This long-lasting activity may originate in the bulb itself, or it may result from local inhibitory interactions in the piriform cortex. To distinguish between these possibilities, we are currently combining cortical feedback imaging to varying odor pulse durations with pharmacological blocking of intracortical interactions.

The enhanced and suppressed bouton responses to a particular odor appeared to be clustered in spatial domains. However, pairwise analysis of simultaneously imaged boutons revealed functional local diversity across our panel of 20 odors. No spatial organization was apparent in bouton responses across odors within the imaged field of view (<150 µm). Enhanced and suppressed boutons also had different population responses to increases in odor concentration; the number of enhanced boutons that responded to an odor increased on average with odor concentration, whereas the number of suppressed boutons followed a nonmonotonic trajectory.

To directly determine the effect of cortical feedback on the dynamics of the OB output, we are suppressing piriform cortex activity using pharmacological and optogenetic methods, in conjunction with simultaneous monitoring of granule and mitral cell activity via multiphoton microscopy. Preliminary results show that suppressing corticofeedback bidirectionally alters spontaneous activity and decreases the responsiveness of granule cells to odors and across concentrations, diminishing both enhanced and suppressed responses.

We propose that cortical feedback modulates the dynamics of OB output such as to sharpen odor responses and maintain diverse neuronal representations across different stimuli. We are currently testing this hypothesis in behaving mice engaged in odor discrimination tasks.

Monitoring Cholinergic Input in the Olfactory Bulb in Mice Engaged in Attention Tasks

Cholinergic inputs from the basal forebrain have been shown to enhance encoding and discrimination of visual, auditory, and somatosensory cues by modulating attention and learning processes. Although rich cholinergic projections innervate the mouse olfactory bulb (MOB), to date, little is known about their contribution to olfactory behaviors. Pharmacological blockade of acetylcholine (ACh) action has been shown to impair olfactory behavior, whereas augmenting ACh levels improved odor discrimination. A clear understanding of the underlying mechanisms of ACh action and their spatiotemporal statistics, however, has been limited by the low yield of recordings from the basal forebrain, as well as difficulties in ascertaining the downstream targets of the recorded neurons, given the widespread and long-range nature of cholinergic projections.

Behavioral Readout

We are using behavioral tasks that require the subjects to identify a stimulus of interest in two different contexts and investigating cholinergic fiber dynamics.

- *Sustained attention task.* In this task, water-deprived mice are trained to discriminate a target odor cue from a nontarget odor to obtain a water reward (Fig. 5A,i). Both the target and nontarget odors are presented at different intensities and timing and, more importantly, in the presence of a varying background odor. To maintain a high success rate, the animals are required to learn to maintain attention after the background odor is presented and detect the onset of the target odor, despite the fluctuating signal to noise across trials. In this context, we are monitoring whether cholinergic activity is time-locked to the background presentation and persists until target/nontarget presentation. Furthermore, we analyzed whether the levels of cholinergic input correlate with trial-to-trial success. Informed by these results, we aim to manipulate cholinergic inputs (optically and reversibly), selectively, in either the OB or medial prefrontal cortex (mPFC) to verify the causal mechanisms that underlie attentional strategies in this behavioral paradigm.
- *Selective attention task.* In this task, mice are trained to discriminate between a target and nontarget odor, as well as a target and nontarget sound stimulus (tone). After this basic learning phase, both olfactory and auditory cues are presented simultaneously, but the availability of reward is locked only to one pair of cues. This reward contingency is switched randomly across blocks of contiguous trials (odor block, sound block) (Fig. 5A,ii). In this

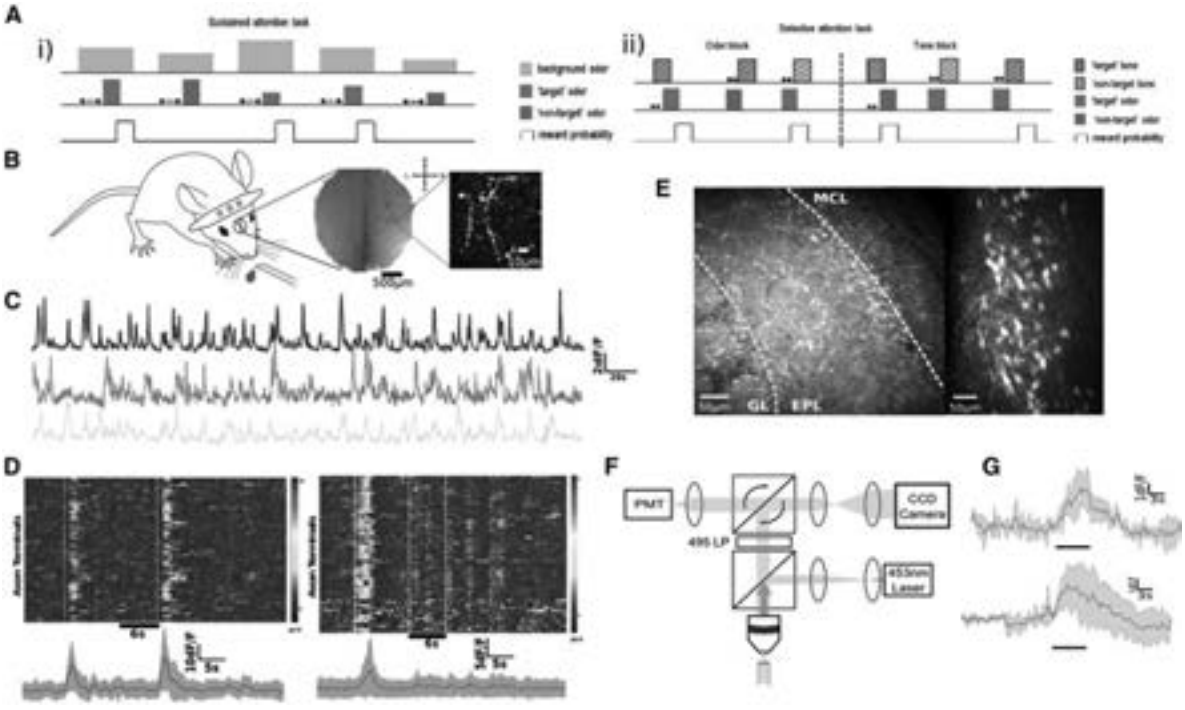

Figure 5. (*A*) (*i*) Sustained attention and (*ii*) selective attention tasks. (*B*) 1-mon-old cranial window over MOB, cholinergic axons expressing GCaMP5 imaged at 100-µm depth (150 × 150-µm field of view). (*C*) Recorded traces spontaneous activity of cholinergic axons form three different ROIs (4 min, mean dF/F_0 = 200%). (*D*) Two examples of cue-evoked (white lines) transients of cholinergic axon activity in awake animals. Rows represent different axon boutons. (*Bottom*) Mean (black line) and standard deviation (gray line) of all responsive axon boutons (mean dF/F for transients = 200%). (*E*) Spread of cholinergic axons expressing GCaMP5 in different layers of the MOB, injection site showing cholinergic cell bodies. (*F*) Diagram of the fiber-optic imaging setup. (*G*) Two proof-of-principle odor-evoked responses acquired with optical fibers from cortical feedback axons expressing GCaMp3 (*left*, 6-sec odor [black bar], mean dF/F0 = 3%, four repeats each) recorded in the main olfactory bulb.

context, we are investigating if and how the cholinergic system enables the subject to selectively direct attention to odor or sound cues. This will allow us to understand the general principles that underlie ACh-mediated attentional processes, whether attention signals are broadcasted as global "upstates" throughout the brain, or whether, depending on the task requirement, only selective brain areas receive modulatory inputs. The latter analysis will provide critical insight into whether excess/deficit of cholinergic activity impairs brain function, particularly under conditions of sensory overflow.

To circumvent low yield of electrophysiological recordings, we are taking advantage of genetically engineered mice (Chat-Cre) and viral strategies to target expression of calcium indicators (GCaMP5 and GCaMP6) in cholinergic neurons and optically monitor the activity patterns of the projection fibers to the MOB in behaving animals (Fig. 5). We are pursuing two complementary strategies to gain access to the activity of ensembles of cholinergic neurons: (1) multiphoton imaging in awake head-fixed mice (Fig. 5B–E), which allows observation of a large number of cholinergic projections in the MOB, with axonal resolution, enabling precise spatiotemporal characterization of their activity patterns within, as well as across, different circuit layers (glomerular versus mitral cell layer), and (2) fiber-optic imaging in freely moving mice (Fig. 5F,G). Although limited to assessment of average population activity of cholinergic inputs, this approach can be easily coupled with electrophysiological means to simultaneously monitor the OB output (M/T cells).

Combining insights obtained via these two approaches will reveal how ACh action in the OB is linked to the timing and nature of olfactory stimuli across different behavioral states. In addition, it will further aid informed optogenetic manipulations of ACh signals to

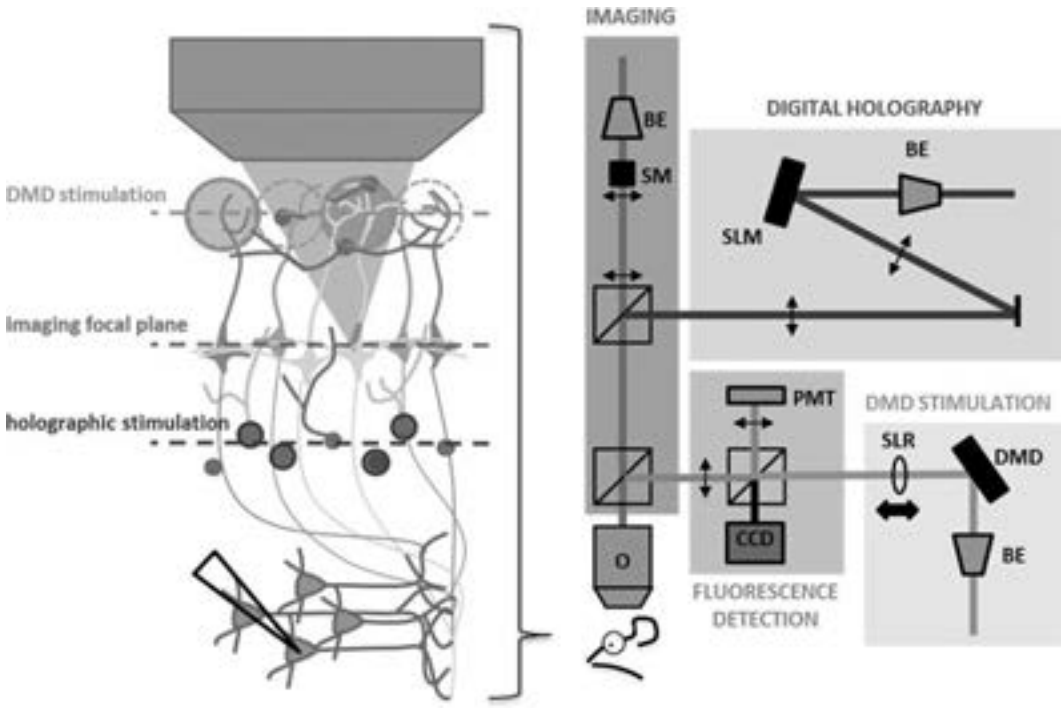

Figure 6. Combined imaging and photostimulation setup. (*Left*) Experimental configuration, combining scanning two-photon imaging (red), DMD photo-stimulation (blue), and holographic photo-stimulation (brown). (*Right*) Schematic of the experimental microscope. DMD stimulation is used to create spatiotemporal light patterns on the surface (<100 µm). Digital holography is used to photo-stimulate deeper (<500 µm) in the brain with cellular resolution. Calcium activity is monitored in an independent optical plane by two-photon imaging and by electrodes in downstream brain regions. (BE) Beam expander; (SM) scan mirrors; (O) objective; (PMT) photo-multiplier; (SLR) camera lens; (DMD) digital micro-mirror device; (SLM) spatial light modulator; (CCD) charged coupled device.

elucidate the relevance of cholinergic inputs in shaping mitral cell output and olfactory behaviors.

Implementing Digital Holography Methods to Investigate in a Closed-Loop Fashion the Spatiotemporal Integration Rules in the Olfactory Bulb and Cortex

We are implementing strategies that will enable non-invasive functional dissection of neuronal networks, with cellular resolution, in behaving animals. This will be brought about via a closed-loop strategy involving real-time control of activity of select neurons with simultaneous monitoring of the concomitant effects of these manipulations on neuronal outputs within the circuit, and elsewhere in the brain. Briefly, we are using digital holography methods via spatial light modulators to optogenetically control neurons of interest at the single-cell level and DMD-based methods to control cell-type-specific populations across large brain regions (Fig. 6). This allows us to both replicate and systematically manipulate stimulus-evoked activity patterns in a circuit. We are simultaneously using two-photon calcium imaging and electrophysiology within the same and different brain regions (olfactory bulb vs. olfactory cortex) to dissect how the alteration of select circuit elements, or their specific properties, affects the output of the network. This closed-loop approach will make it possible to determine the spatiotemporal integration rules within the bulb and the olfactory cortex, investigate the relevance of spike-time codes, and reveal underlying decoding schemes.

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); sequencing the OB—bridging the gap between glomerular odor responses and odor receptor sequences by identifying the molecular identity of glomeruli (A. Koulakov, G. Hannon, and A. Zador); a fiber-optic-based approach to monitor neuronal activity in punishment and reward neuronal circuits during behavior (B. Li) (we will focus on monitoring activity in the insula cortex and basal layer); developing light-sheet-based approaches for fast optical reconstruction of neuronal circuits (P. Osten); and optical monitoring and manipulation of neuronal activity in genetically and anatomically defined cortical circuits in animal models of cognition (A. Zador).

INVESTIGATING NEURAL CIRCUITS FOR SENSORY INTEGRATION AND DECISION-MAKING

A. Churchland A. Brown, O. Odoemene, M. Kaufman, M. Ryan, D. Nunes Raposo, 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 that 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. Impairments in multisensory processing are also observed in subjects with a collection of sensory abnormalities known together as sensory processing disorder and 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 projects in previous years constituted the very first steps toward achieving this goal: We developed a multisensory behavior that could be carefully measured in both humans and rodents. A second publication about this foundational work was published this year (Sheppard et al. 2013a). In addition, we built on this foundation by measuring the responses of neurons in rodents engaged in the behavior, affording insight into the neural circuits underlying multisensory decisions.

The Role of Parietal Cortex in Multisensory Decision-Making

This work was done in collaboration with D. Raposo (Champalimaud Neuroscience Program, Lisbon, Portugal), M. Kaufman (Stanford University), and J. Sheppard (Watson School).

The goal of this project is to gain a deeper understanding of the neural circuits that enable integration of visual and auditory inputs for decision-making. David Raposo has been a leader in the lab in two techniques this year: He measures electrophysiological responses from neurons in the posterior parietal cortex (PPC) of rats engaged in decision-making behavior. He presented this work at two conferences (Raposo et al. 2013). In addition, he inactivated those same neurons and measured the effect on behavior (Raposo and Churchland 2013). Matt Kaufman has worked alongside David to develop sophisticated analyses of

the data. John Sheppard has also contributed to the project by recording additional neurons that are included in our growing population of cells. John, David, and Matt are working as a group; their main finding is that PPC neurons reflect random combinations of stimulus features, but they can be decoded at the population level to provide the animal with ongoing estimates of incoming sensory stimuli. The three are working collectively to write up the exciting results from these experiments for publication.

Population Dynamics Across Cortex

This work was done in collaboration with M. Kaufman (Stanford University).

The goal of this project is to understand how population activity changes from one neural structure to the next to support behavior. To tackle this question, Matt has brought a new technique to the lab: two-photon imaging. Using this technique, we can measure the responses of 80 to 100 neurons simultaneously. This approach will revolutionize the kinds of questions we can address about decision-making, especially when used in conjunctions with emerging mathematical techniques for analysis. We have benefited greatly in this project from technical support from the Albeanu lab, our neighbors in the Marks building, and valued collaborators.

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

This work was done in collaboration with J. Sheppard (Watson School of Biological Sciences).

The goal of this project is to understand how the brain estimates the reliability of sensory stimuli and uses that estimate to guide decision-making. John published a paper on his behavioral data relevant to this question this year (Sheppard et al. 2013a), demonstrating to the field that rats are capable of optimally weighting sensory information to guide behavior. John's results were a surprise to many skeptics who doubted that rodents were capable of such sophisticated behavior. He has also led the lab in using an optogenetic approach to understand how the posterior parietal cortex (PPC) drives behavior. He presented this work at the Society for Neuroscience Annual Meeting (Sheppard et al. 2013b) and is continuing to collect data.

Decision-Making in Mice

This work was done in collaboration with O. Odoemene (Watson School of Biological Sciences).

We have developed a decision-making paradigm using mice that will allow us to take advantage of genetic tools. These tools will also allow us to probe the neural circuitry that underlies decision-making. In the past year, Onyekachi Odoemene has sought to establish which neural structures in mice are critical for decision-making. He is using cutting-edge techniques to inactivate different structures and examine the effect on behavior. By using different strains of transgenic mice, Odoemene is able to target particular cell types within each area and examine how they contribute to behavior. This project had benefited greatly from our collaborator Dr. Z. Josh Huang, who has provided us with materials for experiments, as well as invaluable advice on understanding inhibitory circuitry.

Bayesian Number Estimation

A. Brown

This project aims to understand whether humans use a probabilistic approach to number estimation, and it is part of an international collaboration with Dr. Alexandre Pouget (University of Geneva). This work challenges a long-standing assumption of human number estimation—that numbers are estimated as scalar quantities. Our experiments suggest that the assumption is not correct. Instead, humans appear to represent numbers probabilistically, and a signature of this representation is evident when they combine number estimates from two modalities. Amanda copresented this work at the Society for Neuroscience Annual Meeting (Kanitscheider et al. 2013), and we are writing up this work for publication now.

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MECHANISMS OF NEURODEGENERATION AND MEMORY FORMATION

J. Dubnau R. Borges Monroy, N. Chatterjee, M. Cressy, W. Donovan, L. Krug, W. Li, L. Prazak, H. Qin, M.-F. Shih

There are two main areas of research in my lab. First, we are using neurogenetic and genomics approaches to investigate a novel hypothesis to explain neurodegenerative disorders such as frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Second, we are investigating the genetic and neuronal mechanisms of memory formation using *Drosophila* as a model system.

Impact of Retrotransposons on Aging and Neurodegeneration Underlying Frontotemporal Lobar Degeneration and Amyotrophic Lateral Sclerosis

R. Borges, N. Chatterjee, W. Donovan, L. Krug, L. Prazak, J. Dubnau [in collaboration with M. Hammell, Y. Jin, T. Zador, Cold Spring Harbor Laboratory]

Transposable elements (TEs) 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 (endogenous retrovirus) expression may contribute to TDP-43-mediated neurodegenerative disorders (Li et al. 2013). We have found that TE transcripts are derepressed during the normal aging process and that this leads to active mobilization of transposons, resulting in new insertions in the genome of neurons (Fig. 1). 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 lifespan. These findings support the hypothesis that protracted loss of TE silencing contributes to age-dependent decline in neuronal physiological function.

To investigate the hypothesis that TE derepression contributes to neurodegenerative disorders, we focused (in collaboration with Molly Hammell and Ying Jin) on TDP-43, an RNA-binding protein involved in a broad spectrum of neurodegenerative disorders including amyotrophic lateral sclerosis (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 (Li et al., *PloS One 7:* e44099 [2012]). We also find that association between TDP-43 and many of its TE targets is reduced in FTLD patients (Fig. 2). Finally, we discovered that a large fraction of the TEs to which TDP-43 binds become derepressed in mouse TDP-43 disease models. We propose the hypothesis that TE misregulation contributes to TDP-43-related neurodegenerative disease. Current efforts are focused on dissection of the underlying mechanisms of transposon control in the brain and the role of unregulated transposon expression in these disorders. Efforts involve work in *Drosophila* and mouse models of TDP-43 pathology, in human cell culture and in postmortem tissue from human subjects with FTLD or ALS.

Age-Dependent Memory Impairment and Retrotransposon Activation

L. Prazak

On the basis of our observation that certain retrotransposons become highly active during normal brain aging, we are investigating the hypothesis that retrotransposon-generated DNA damage contributes to age-related memory impairment. Using the toolbox for genetic manipulations that are available in *Drosophila*, we are (1) investigating the normal mechanisms of retrotransposon silencing in the brain, (2) testing the impacts of age on these cellular mechanisms, and (3) testing whether retrotransposon activation has a causal role in age-related cognitive impairment.

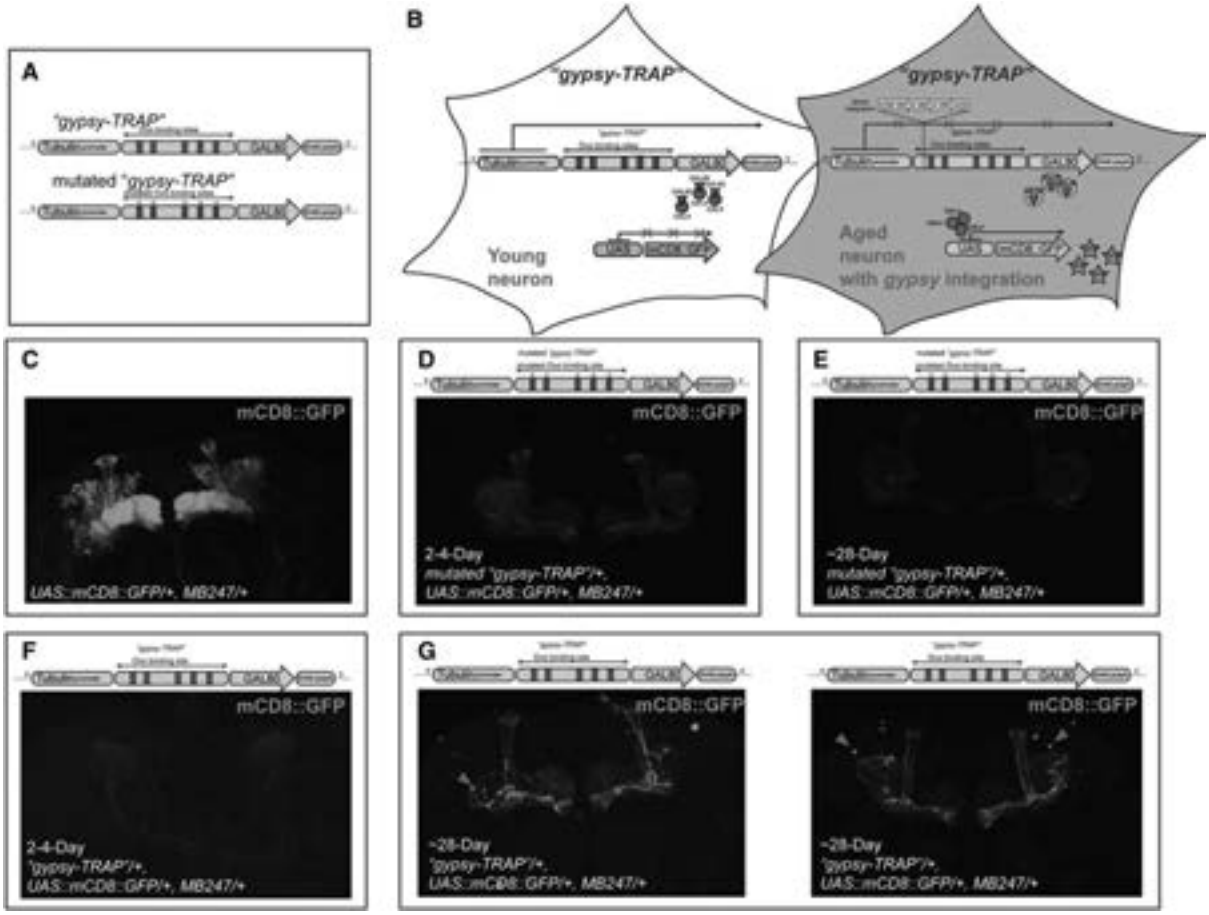

Figure 1. *"Gypsy-TRAP"* reporter detects de novo integration in neurons in aged animals. (*A,B*) Illustration of the design of *"gypsy-TRAP."* An ~500-bp fragment from the *ovo* regulatory region containing five Ovo-binding sites is inserted between the *Tub promoter* and *GAL80* gene (*A*). A mutated *"gypsy-TRAP"* construct contains mutations that disrupt each of the five Ovo-binding sites. (*B*) In the absence of *gypsy* insertions, GAL80 expression suppresses GAL4, and *UAS::mCD8::GFP* expression does not occur. In the presence of *gypsy* integration into the *"gypsy-TRAP,"* GAL80 expression is blocked, and *UAS::mCD8::GFP* is turned on. (*C*) Approximately 800 MB Kenyon cell neurons per brain hemisphere are labeled by *MB247-GAL4*-driven *U*AS::mCD8::GFP. (*D*) An example brain from 2–4-day-old mutated *"gypsy-TRAP"; UAS::mCD8::GFP/+; MB247/+*. No green fluorescent protein (GFP)-labeled neurons were seen. (*E*) An example brain from ~28-day-old mutated *"gypsy-TRAP"*; *UAS::mCD8::GFP/+; MB247/+*. No GFP-labeled neurons were seen. (*F*) An example brain from ~2–4-day-old *"gypsy-TRAP"*; *UAS::mCD8::GFP/+; MB247/+*. No GFP-labeled neurons were seen. (*G*) Example brains from ~28-day-old *"gypsy-TRAP"; UAS::mCD8::GFP/+; MB247/+*. Several GFP-labeled MB neurons were seen in each brain.

Neurogenetic Mechanisms of Olfactory Memory

L. Prazak, M.F. Shih

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.

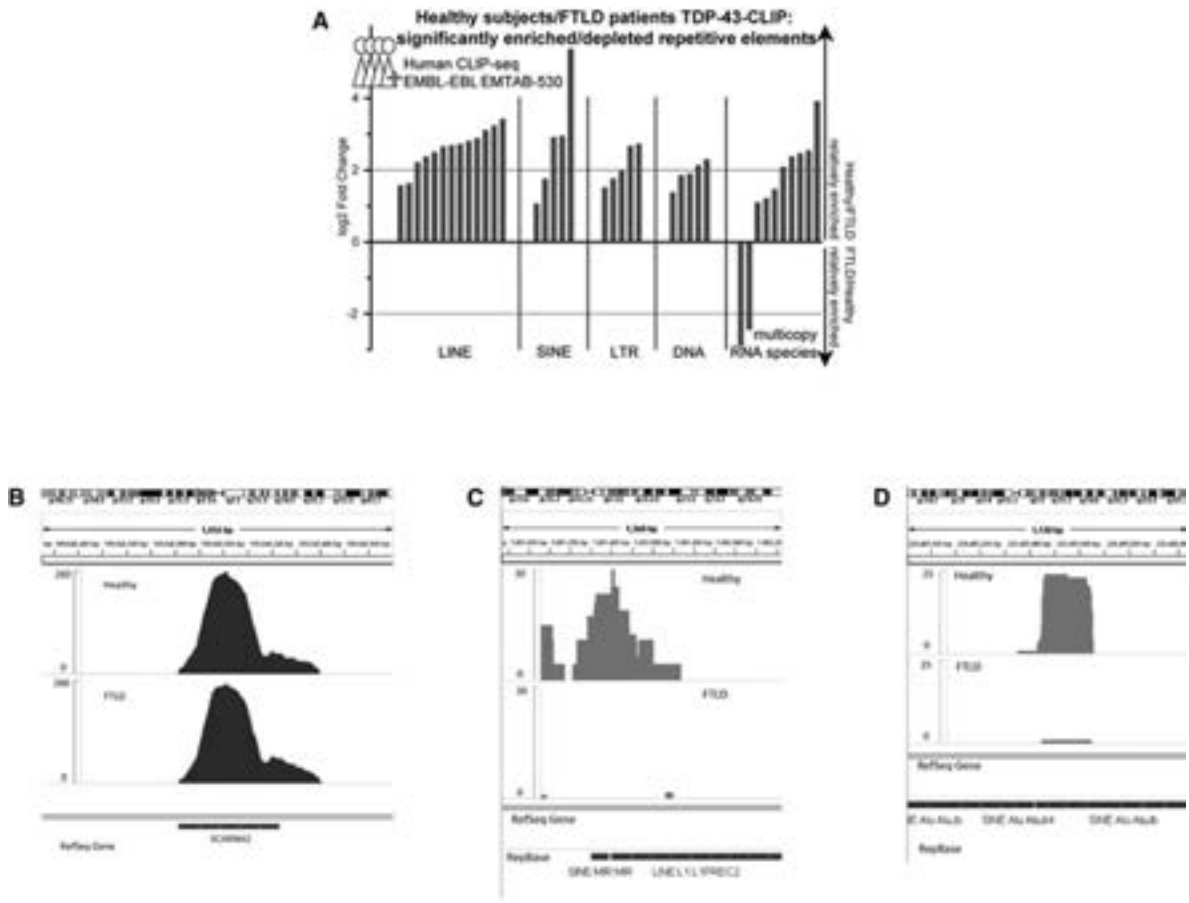

Figure 2. TDP-43 binding to TEs is selectively lost in FTLD patients. (*A*) In the human CLIP-Seq data from FTLD versus healthy control, 38 repeat elements showed significant (p-value ≤ 1e-5 and fold changes ≥ 2) differential binding. Log2-fold binding differences are shown for significantly enriched/depleted elements. (*B,C,D*) Peaks are shown in genome browser for one RefGene control (*B*) and two differentially targeted TEs (*C,D*) in healthy (*top*) versus FTLD (*bottom*).

A MicroRNA–Dopamine Receptor Genetic Module in Distinct Neural Circuits for Olfactory Arousal and Olfactory Memory

W. Li, M. Cressy, H. Qin

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 (Li et al. 2013b). In both circuits, the miR 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 neurons to support long-term olfactory memory and in 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 (MB) and central complex.

Cell-Type-Specific Expression Profiling to Identify Memory-Related Targets of miR276a

M.F. Shih

We have demonstrated a role for a miRNA gene, miR276a, in olfactory memory in *Drosophila*. Our findings implicate this miRNA in two different neuronal cell types (mushroom body Kenyon cells and a subset of the so-called ellipsoid body neurons). We are using methods for cell-type-specific expression profiling to identify the relevant targets of miR276a within each of these neuronal cell types.

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STEM CELLS, SIGNAL TRANSDUCTION, AND BRAIN FUNCTION

G. Enikolopov Z. Glass T. Michurina J.-H. Park O. Podgorny
R. Makayus O. Mineyeva N. Peunova

Our main focus is on stem cells of the adult organism. Our research aims to identify stem cells in different tissues, understand the molecular logic of their maintenance and differentiation, and apply this knowledge for human therapy. Our main approach is generating animal models that would allow visualization of stem cells and their environment and monitoring changes in their signaling landscape. Although most of our work is related to adult neural stem cells, we are also investigating stem cells and regulatory signals in non-neural tissues, particularly those that are involved in complex physiological circuits of the organism.

Neural Stem Cells, Aging, and Brain Disorders

The key focus of our group is on adult neurogenesis, maintenance of adult neural stem cells, and signals that control them. Emerging evidence indicates that adult neurogenesis is important for behavior, pathophysiology, aging, and neural tissue repair; therefore, a clear understanding of the rules that govern the maintenance, division, and differentiation of adult neural stem cells may have direct implications for human aging, disease, and therapy. We recently showed that age-related decrease in hippocampal neurogenesis under normal conditions is driven by the disappearance of neural stem cells via their conversion into mature hippocampal astrocytes. We described the life cycle of an adult neural stem cell and proposed a "disposable stem cell" model which posits that an adult hippocampal stem cell is quiescent throughout the entire postnatal life, but, when activated, it undergoes several rapid asymmetric divisions (eventually producing a neuron) and then exits the pool of stem cells by converting into an astrocyte. Our model reconciles observations on the age-related decrease in new neurons and age-related increase in astrocytes, the disappearance of hippocampal neural stem cells, and the remodeling of the neurogenic niche. These continuous changes underlie age-dependent diminished production of new neurons and may contribute to age-related cognitive impairment.

We also used our reporter lines and our model to determine the classes of stem and progenitor cells in the hippocampus that are affected by various pro- and antineurogenic factors and stimuli. This included factors that increase hippocampal neurogenesis, such as SSRI and SNRI (selective serotonin and norepinephrine reuptake inhibitors) antidepressants, electroconvulsive shock, deep brain stimulation of the anterior thalamic nucleus and cingulate gyrus, and physical exercise. Our model of adult hippocampal neurogenesis predicts that specific treatments may accelerate production of new neurons by various means (e.g., via increased division of stem cell progeny, increased symmetric or asymmetric division of stem cells, increased recruitment of quiescent stem cells, or suppression of cell death) and that each mode of augmented production of new neurons may have different effects on the pool of stem cells. Indeed, we found examples of each of the different modes of activation of neurogenesis in response to specific stimuli (so far, except for symmetric division of stem cells). Importantly, we found examples of increased recruitment of normally quiescent stem cells in division induced by a widely used therapeutic drug; accompanied by astrocytic differentiation, such recruitment would lead to an increased number of new neurons but at the expense of premature exhaustion of the stem cell pool. In contrast, we found that electroconvulsive shock increases the number of asymmetric divisions of stem cells without recruiting additional stem cells, therefore leading to an increased number of new neurons without additional loss of stem cells. Thus, these experimental studies of adult neurogenesis may have direct relevance to human therapy.

Much of our interest is related to the effect of aging on stem cells. Lifespan of a diverse range of species can be significantly extended by restricting the calorie intake or by treatment with the mTOR (mammalian target of rapamycin) pathway inhibitor rapamycin. Both calorie restriction (CR) and rapamycin have also

been shown to affect adult neurogenesis. These treatments are usually applied for a limited period of time, and it is not clear whether prolonged treatment with CR or rapamycin has similar benefits and whether such benefits are observed in old animals. It is also not clear which specific subclasses of neural stem and progenitor cells and which steps of the neuronal differentiation cascade are targeted by CR or rapamycin. We used our reporter mouse lines to investigate the effects of CR and rapamycin on hippocampal stem and progenitor cells in aging animals and found that prolonged (12- or 18-mo) exposure of animals to CR (but not to rapamycin) alleviates age-related decrease in neural progenitor cell division in the aging brain; the increase in the number of dividing cells was particularly evident in the hippocampus of female mice. Our results indicate that the majority of the dividing cells correspond to neural stem and progenitor cells and that CR may increase the number of divisions that neural stem and progenitor cells undergo in the aging brain of female animals.

Much of our effort is related to developing new methods to study adult neurogenesis. One such project concerns three-dimensional representation of neural stem cells in the adult brain. There have been several attempts to obtain a three-dimensional representation of the stem cell compartments in the adult brain; however, they were either too labor-intensive or imprecise. We have applied a new method of serial two-photon tomography to our reporter mouse lines to produce a quantitative three-dimensional atlas of stem and progenitor cells in the adult brain (in collaboration with Dr. Pavel Osten, CSHL). The resulting image shows active stem and progenitor cells, as well as cells with stem cell potential, in the regions of robust neurogenesis, the circumventricular organs, and the central canal; importantly, such imaging is quantitative by the nature of the technique. This approach may become a powerful tool to analyze stem cells upon aging, in behavioral paradigms, or in the context of disease.

Another project concerns triple S-phase labeling to increase the resolution of the cell cycle analysis. Labeling cells undergoing the S phase of the cell cycle with a nucleotide analogue, such as bromodeoxyuridine (BrdU) or ethyldeoxyuridine (EdU), became the main approach to analyze cell division, stem cell division in particular. This method has been greatly improved by the ability to double-label the S phase using pairs of modified nucleotides, e.g., chlorodeoxyuridine (CldU) and iododeoxyuridine (IdU) or BrdU and EdU. We have now developed a new method for labeling dividing stem and progenitor cells, based on sequential labeling with three different nucleotide analogues on the background of green fluorescent protein (GFP)-labeled cells (i.e., quadruple labeling). Our method allows a qualitatively new level of resolution of cell division kinetics: tracing of multiple cell populations in a normal, transformed, damaged, or regenerating tissue and tracing of progeny of multiple stem and progenitor cell subpopulations. We are currently applying this new approach to determine the changes in neural stem cell maintenance, division, and differentiation in the aging hippocampus and in response to drugs.

We also continue our collaboration with Dr. Helene Benveniste (Stony Brook University) on metabolic profiling of dividing cells in the live rodent brain using proton magnetic resonance spectroscopy. We apply a widely used spectral fitting algorithm (LCModel) to characterize the effect of increased progenitor cell division in the model of electroconvulsive therapy and in a mouse model of glioblastoma. Our results indicate that this method can detect characteristic lipid signatures at high concentrations of dividing neural progenitor-like cells in live animals.

Stem Cells in Nonneural Tissues

Much of our effort is based on using engineered reporter mouse lines to visualize, track, and isolate stem cells. We originated this approach more than 15 years ago, and some of our reporter lines (e.g., Nestin-GFP mice) became popular and useful genetic tools used by many laboratories to study stem cells. Although these lines were originally designed to identify stem cells in the nervous system, we soon found that the same reporter lines highlight stem and progenitor cells in a range of other tissues. These tissues are as diverse as anterior pituitary, skeletal muscle, testis, hair follicles, liver, pancreas, and bone marrow.

We have been expanding on the reporter approach by generating double- and triple-reporter lines, based on various regulatory elements, including those that use promoters of *Sox2, Gli1,* or *Lgr5* genes (e.g., *Sox2*-GFP/Nestin-mCherry or *Lgr5*-mCherry/Nestin-GFP). Using this approach, we discovered a new population

of stem cells in the ovary that normally act to repair the epithelial surface of the ovary but that can be easily transformed and become cells of origin of ovarian tumors (a collaboration with Dr. Alex Nikitin from Cornell University). When an oocyte leaves the ovary during ovulation, the ovarian surface epithelium is ruptured and has to be repaired. We found a small population of epithelial cells in the mouse ovary that have the main characteristics of stem cells: They express several characteristic markers of stem cells, they are mainly quiescent, and their progeny can differentiate and repair the surface epithelium after the oocyte exits. These stem cells show increased propensity to be transformed after inactivation of tumor suppressor genes *p53* and *Rb1*, whose pathways are altered frequently in the most aggressive and common type of human ovarian cancer, high-grade serous adenocarcinoma. Thus, we found a new type of stem cells and extended support for the often discussed notion that susceptibility of transitional zones between different types of epithelia (of which the epithelial cells of the ovarian hilum are an example) to malignant transformation may be explained by the presence of stem cell niches in those areas.

Signaling in the Stem Cell Niche

One of the our new directions is elucidating the signaling landscape of the neurogenic regions in the adult brain. Our current focus is on redox signaling, which emerges as an important regulator of stem cell maintenance. The changes in the redox status and production of reactive oxygen and nitrogen species (ROS) change the proliferation and differentiation of various classes of stem and progenitor cells, including neural cells. Aging correlates with increased production of ROS, and this increase may be crucial for various manifestation of aging. We develop genetically encoded sensors that report different aspects of redox signaling and apply them to visualize signaling processes in and around aging neural stem cells and their subcellular compartments, with a larger goal of tying these processes to normal aging or disease (much of this work is carried out in collaboration with Dr. Vsevolod Belousov, a Visiting Scientist in the group). Among other results, we have (1) generated and validated a new sensor for imaging changes in the NAD^+/NADH ratio in various cellular compartments (e.g., mitochondria); (2) developed a method for controlled production of ROS in mammalian cells and parallel measuring the levels of produced ROS and demonstrated that the efficiency of redox-sensitive oxidation of cysteine residues depends on the proximity to the source of H_2O_2; and (3) generated and validated a red recombinant redox sensor. We are now working on expanding the panel of sensors and reporters for parallel visualization of multiple signaling events controlling the stem cell niche.

NO, Development, and Differentiation

During the last decade, we discovered an essential role of nitric oxide (NO) in development and in stem cell regulation. We continue to uncover the diversity of biological functions mediated by NO. For instance, we found that NO is an important mediator of activity-dependent neuronal development in the hippocampus, promoting local activity-dependent spine growth (collaboration with Dr. Dominique Muller, University of Geneva). Ablation of activity of neuronal NO synthase (NOS) interferes with the development of excitatory synapses and prevents structural adaptation of hippocampal excitatory synapses to environmental enrichment.

We found yet another important function of NO: its critical role in the multiciliated cells of the mucociliary epithelium. Cilia of these cells beat in concert to generate directed flow of fluid across tissue; in the trachea and bronchi, this flow helps to clear pathogens, foreign particles, and toxic chemicals from the airways. We found that this role of NO is conserved, and its basic features are highly similar in such diverse settings as skin of the frog embryo and mammalian airways. We identified distinct NO-dependent signaling pathways that control the cilia distribution, cilia polarity, and cilia length. Our results suggest that some of the inborn and acquired human ciliopathies and related disorders may also be associated with inadequate activity of NOS and decreased availability of NO and may thus benefit from NO-based therapies.

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MOLECULAR ANALYSIS OF NEURONAL RECEPTORS AND ION CHANNELS

H. Furukawa E. Karakas, T. Malinauskas, A. Romero Hernandez, N. Simorowski, N. Tajima

The research program in our lab attempts to understand the molecular basis of the functions of the cell surface receptors or membrane proteins that initiate cellular signal transductions involved in neurotransmission in the mammalian brain, in order to develop compounds with therapeutic value for treating neurological diseases and disorders including schizophrenia, depression, stroke, and Alzheimer's disease. Toward this end, we are conducting structural and functional studies on ion channels that control intracellular calcium signaling upon stimulation by voltage and/or neurotransmitters. These ion channels regulate the strength of neurotransmission, the fundamental process whereby multiple neurons communicate with one another. Dysfunction of the ion channel studied in our group is strongly implicated in neurotoxicity that results in various neurological disorders and diseases described above. To achieve our goals, we use X-ray crystallography to determine three-dimensional atomic structures of target proteins and test structure-based mechanistic hypotheses by site-directed mutagenesis in combination with biochemical and biophysical techniques. The main accomplishment in 2013 was the completion of high-resolution structures of the ligand-binding domain (LBD) of a ligand-gated ion channel, *N*-methyl-D-aspartate receptor (NMDAR) in complex with antagonists and validation of the crystal structures by electrophysiological techniques. The crystal structure shows the overall change in protein conformation within LBD upon binding to two types of antagonists compared to the agonist-bound form. The electrophysiological experiments pinpointed elements possibly responsible for subtype-specific inhibition of NMDARs.

Structural Studies on the Ligand Binding Domain of NMDAR

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 mainly of two copies each of the GluN1 and GluN2 subunits, which bind glycine and glutamate, respectively. The calcium signals triggered by NMDARs facilitate cellular signal transduction, resulting in neuroplasticity essential for learning and memory formation. Dysfunctional NMDARs are implicated in various neurological disorders and diseases. For example, hypofunction of NMDAR is a well-known paradigm for schizophrenia. Overactivation of NMDARs causes depression and neurodegenerative responses associated with Alzheimer's disease and stroke-induced ischemic injuries. The four distinct GluN2 subunits (A–D) control the pharmacological properties of the NMDAR ion channels and show different spatial and temporal expression patterns; thus, there has been great interest in creating subtype-specific compounds that can target specific neuronal circuits. The NMDAR subunits are modular and are composed of distinct protein domains including the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TMD), and carboxy-terminal domain (CTD) (Fig. 1). Our group has obtained the first crystal structures of GluN1/GluN2 ATD and LBD and provided the field with important structural insights into how subunits may be arranged in those domains in the context of the tetrameric NMDAR ion channels. However, there has not been any detailed information on the binding modes of various inhibitors and competitive antagonists. In 2013, we completed structure/function studies on competitive antagonism on NMDARs mediated by LBD.

Structural information on the antagonist-bound form of NMDAR has thus far been restricted to that of GluN1 LBD. Thus, despite the historical importance, the molecular mechanism underlying inhibition of NMDARs by D-AP5 at GluN2

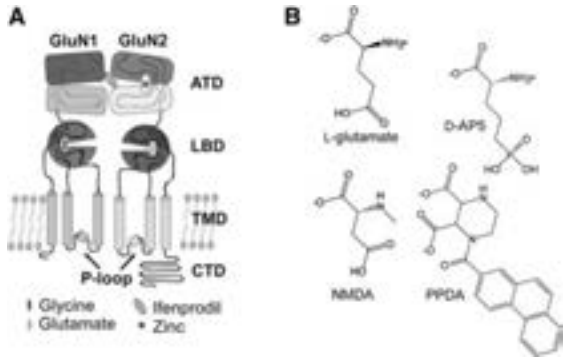

Figure 1. NMDAR subunit organization and ligands. NMDAR subunits are modular protein composed of an amino-terminal domain (ATD), a ligand-binding domain (LBD), a transmembrane domain (TMD), and a cytosolic domain (CTD). The domains are oriented such that the amino terminus (NT) and the carboxyl terminus (CT) are located at the extracellular and cytoplasmic regions, respectively. LBDs from GluN1 and GluN2A are isolated (scissors) by tethering two peptide fragments between TMD M1 and M3 by a Gly-Thr dipeptide linker (dashed line). (*B*) Ligands for GluN2 subunits. L-glutamate and NMDA bind GluN2 LBD. (2*R*)-amino-5-phosphonovaleric acid (D-AP5) and 1-(phenanthrene-2-carbonyl)piperazine-2,3-dicarboxylic acid (PPDA) with distinct chemical structures both act as competitive antagonists at the GluN2 LBD.

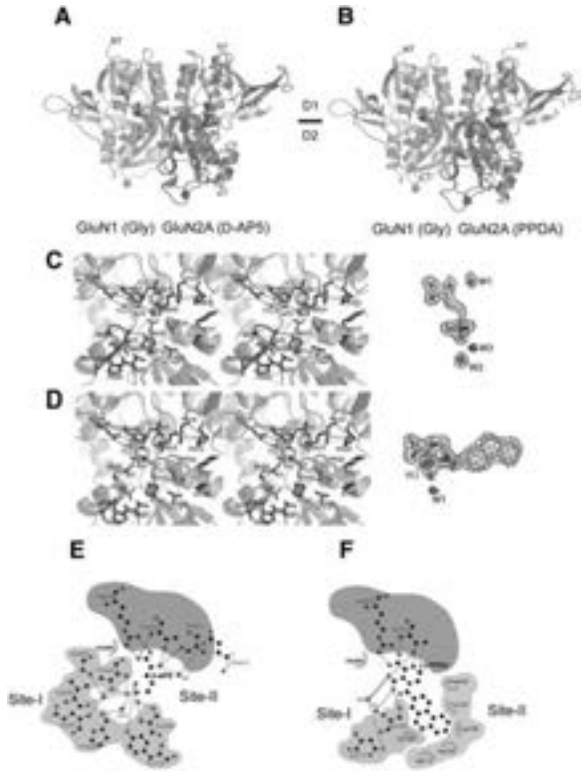

Figure 2. Crystal structures of GluN1/GluN2A LBD in complex with GluN2-antagonists. The GluN1 and GluN2A LBDs form physiological heterodimers in the crystal. Each subunit has bilobed structures composed of upper (D1) and lower (D2) domains. Ligands such as D-AP5 (*A*) and PPDA (*B*) bind at the intercleft between D1 and D2. Stereoview of the binding sites for D-AP5 (*C*) and PPDA (*D*). (*Right panel*) *Fo-Fc* electron density contoured at 4σ. LIG-PLOT presentation of the binding residues for D-AP5 (*E*) and PPDA (*F*). The antagonist binding sites are composed of two subsites called Site-I and Site-II.

LBD has remained enigmatic. Furthermore, how the phenanthrene-based compounds such as PPDA, with a chemical structure highly distinct from that of D-AP5, can function as an antagonist has been a mystery (Fig. 1). These questions have been difficult to answer because of the technical difficulties associated with crystallization of the antagonist-bound GluN2 subunit. We overcame this technical barrier by developing a crystallographic method that involved soaking, which resulted in two crystal structures bound to (1) glycine and D-AP5 and (2) glycine and PPDA.

The study shows that D-AP5 and PPDA bind to the ligand-binding site within the GluN2A LBD in distinct modes and result in "opening" of the bilobed LBD structure to different extents (Fig. 2). Additionally, the study demonstrated that the phenanthrene ring of PPDA forming van der Waals interactions with the hydrophobic pocket and sulfur–aromatic interaction with a methionine residue present in GluN2B-D in Site-II is responsible for the moderate subtype specificity within NMDARs. The work also established an effective method for obtaining crystals that permit visualization of antagonist-bound GluN1/GluN2A LBD structures at high resolution.

Validating Binding Modes of Antagonists

Inspection of the ligand-binding site shows distinct binding modes between D-AP5 and (-)-PPDA involving different residues in the GluN2A ligand-binding site. To validate the physiological relevance of this structural observation and to further understand the chemical nature of the ligand-binding site, we carried out mutational analysis of residues involved in antagonist binding by measuring current inhibition via two-electrode voltage clamp (TEVC). The normalized potencies of D-AP5 and (-)-PPDA were calculated by determining EC_{50} values of L-glutamate and IC_{50} values for D-AP5 and (-)-PPDA at fixed L-glutamate concentrations, and by converting EC_{50} and IC_{50} into K_i values using the Cheng–Prusoff equation for every

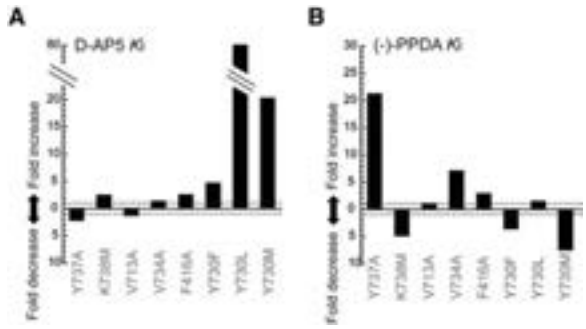

Figure 3. Site-directed mutagenesis of the antagonist binding sites. Residues in Site-I and Site-II (Fig. 2) are mutated in the context of the full-length GluN1/GluN2A NMDARs, expressed in *Xenopus* oocytes, and analyzed for potency. K_i values are calculated based on the EC_{50} values for glutamate and IC_{50} values for antagonists.

mutant tested (Fig. 3). Mutational analysis indeed verified the involvement of distinct residues in binding of D-AP5 and (-)-PPDA and thus validates the physiological relevance of the crystal structures obtained in our current study. In general, mutation of residues surrounding the phenanthrene rings of (-)-PPDA (Fig. 2 in Site-II) affects the potency of (-)-PPDA with little or no effect on the potency of D-AP5. Among these mutations, GluN2A Val734Ala, Tyr737Ala, and Lys738Met have significant effects on (-)-PPDA potency but only minor effects on D-AP5 potency (Fig. 3). An intriguing observation is that although Val734Ala and Tyr737Ala both decrease (-)-PPDA sensitivity by reduction of van der Waals interaction with the phenanthrene ring, Lys738Met increases (-)-PPDA sensitivity by strengthening the interaction (likely via aromatic–sulfur interaction). Among all the GluN2 subunits, GluN2A is the only one with lysine at the 738 position, whereas the other three subunits (GluN2B-D) contain methionine at the equivalent position. GluN1/GluN2A NMDAR is the subtype that is consistently least sensitive to (-)-PPDA. Based on the structural observation above, we propose that the preferential binding of (-)-PPDA toward GluN2B/C/D over GluN2A-containing NMDARs derives from different modes of interaction with the phenanthrene ring at the 738 position. Mutations on the other residues surrounding the phenanthrene ring, Phe416Ala and Val713Ala, have minor effects on sensitivity to both (-)-PPDA and D-AP5, consistent with the structural observation that those residues are farther away from the phenanthrene ring compared to Val734, Tyr737, or Lys738 and, therefore, not at the ideal position to form a strong van der Waals interaction. Tyr730 participates in binding of D-AP5 through polar interaction with the phosphono group and, to a minor extent, of (-)-PPDA through Van der Waals interaction with the piperazine ring. The Tyr730Phe mutation results in a fivefold decrease in sensitivity of D-AP5 by reduction in the number of polar interactions, whereas it results in an increase in (-)-PPDA sensitivity—likely by the strengthening of hydrophobic interactions due to the absence of the hydroxyl group (Fig. 3).

Binding Specificity between NMDARs and Non-NMDARs

Specific inhibition of NMDA-induced currents by antagonists was crucial to confirm the existence of the iGluRs. D-AP5 was one of the first antagonists discovered to specifically inhibit the NMDA-induced current, whereas (-)-PPDA was later reported to inhibit NMDARs with subtype specificity toward GluN2C/GluN2D-containing NMDARs. Structural comparisons between GluN1/GluN2A LBD and non-NMDAR LBDs show elements that may have a role in distinguishing NMDARs and non-NMDARs such as AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and kainate receptors. The residues from D1 in direct contact with or in vicinity of D-AP5 are mostly conserved in non-NMDARs except that the equivalent residue to GluN2A His484 is tyrosine in AMPA and kainate receptors (Fig. 4). As described above, direct and indirect polar interactions in D2 are mediated by side chains of Ser689, Thr690, and Tyr730, and nitrogen and oxygen atoms from the main chain. Although GluN2A Ser689 and Thr690 are conserved in all of the L-glutamate-binding subunits in iGluRs, Tyr730 is unique to the GluN2 subunits in NMDAR family (Fig. 4). The equivalent residues of GluN2A Tyr730 are GluA2 Leu704 and GluK1 Met722, which cannot form direct polar interaction or "cap" the binding site as GluN2A Tyr730 does. Consistent with the structural and primary sequence analyses, mutating GluN2A Tyr730 to leucine or methionine dramatically reduces D-AP5 sensitivity (~20-fold and ~80-fold increase in K_i values in Tyr730Leu and Tyr730Met, respectively), although it causes little or no change in sensitivity to (-)-PPDA. However, mutating GluA2 Leu704 and GluK1 Met722 to tyrosine does not confer sensitivity

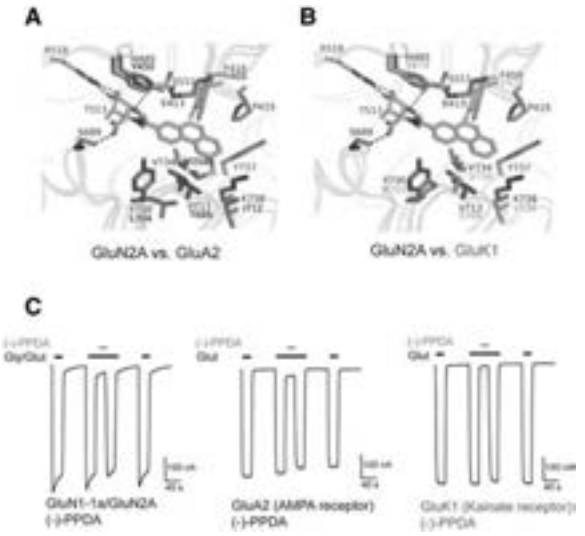

Figure 4. PPDA is capable of recognizing both NMDARs and non-NMDARs. The structural comparison between the GluN1/GluN2A LBD with AMPA receptor (*A*) or kainate receptor (*B*) implicates possible binding of PPDA. Electrophysiological recordings show that every subfamily member of ionotropic glutamate receptors is inhibited by PPDA. Shown here are recordings done in the presence of saturating glycine and glutamate. The current is inhibited by 100 μM of PPDA.

to D-AP5 in these non-NMDARs, indicating that specific binding of D-AP5 to NMDARs is not determined solely by the tyrosine residue in the binding pocket (data not shown). Overall, Tyr730 is a critical factor, but not the only one that facilitates specific inhibition of NMDAR by D-AP5.

Contrary to D-AP5, structural comparison between GluN1/GluN2A LBD and GluA2 or GluK1 LBD shows no apparent molecular feature that may interfere with binding of (-)-PPDA in non-NMDARs, but instead, it indicates a possibility that (-)-PPDA binding will occur (Fig. 4). To validate this structural observation, we assessed the inhibition pattern of (-)-PPDA on L-glutamate-induced currents produced by non-desensitizing mutants of GluA2 Leu483Tyr flip (Stern-Bach et al., [1998]) and GluK1 Tyr506Cys Leu768Cys (Weston et al., [2006]) using TEVC. The application of 100 μM (-)-PPDA in the presence of agonists completely inhibited not only GluN1/GluN2A NMDARs, but also GluA2 AMPA receptors, and GluK1 kainate receptors (Fig. 4). Indeed, both GluA2 and GluK1 respond to (-)-PPDA with K_i values of 7.85 μM and 1.17 μM, respectively, which are comparable to K_i of 0.82 μM in GluN1/GluN2A receptors. These results are consistent with the recent report that some piperazine-2,3-dicarboxylate derivatives can act on both kainate and NMDARs. The current study clearly shows that (-)-PPDA is a general antagonist that acts on all of the L-glutamate-binding iGluR subunits. Unlike PPDA, D-AP5 has no effect on non-NMDARs in similar experiments. This indicates that the interaction between the phosphono group of D-AP5 and Site-I is the crucial determinant for NMDAR specificity over non-NMDARs. Although Site-II does not define NMDAR specificity, it distinguishes different subtypes within the NMDAR families (e.g., GluN1/GluN2A vs. GluN1/GluN2D). Thus, a compound that interacts with both Site-I and Site-II would be a good lead for development of subtype-specific antagonists.

Overall, our work shows the detailed molecular mechanism of antagonist binding in NMDARs and suggests a strategy to develop compounds specific for GluN1/GluN2A subtypes. There has been enthusiasm for such compounds in recent years because of the involvement of GluN1/GluN2A NMDARs in neuropathic pains, fear, and early onset epileptic encephalopathy.

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ASSEMBLY AND FUNCTION OF NEURAL CIRCUITS IN THE NEOCORTEX

Z.J. Huang M. He, D. Huilgol, S. Kelly, K. Krishnan, J. Levine, J. Lu, A.P. Tucciarone, P. Wu

We aim to understand the developmental assembly and functional organization of neural circuits in the cerebral cortex. We have pioneered the use of mouse engineering toward a genetic dissection of GABAergic inhibitory circuitry in the neocortex. In the past year, we extended this effort to targeting distinct classes of excitatory glutamatergic pyramidal neurons. Genetic targeting of distinct neuron types establishes entry points for studying cortical circuits and builds a solid middle ground that coherently connects to system neuroscience, on the one hand, and molecular and developmental neuroscience, on the other hand. In addition, we have been developing strategies for cell-type-based genomic analysis in brain tissues. Our goal is to discover the genetic principles underlying the assembly and organization of cortical circuits and provide insight into how altered development of these circuits contributes to mental disorders.

Genetic Targeting of Pyramidal Neuron Subtypes in the Mouse Neocortex

A key obstacle to studying the development, organization, and function of neural circuits in the cerebral cortex is the stunning diversity of neuron types and a lack of comprehensive knowledge about their basic biology. The problem of neuronal diversity and identity in the cortex is fundamental, transcending developmental and systems neuroscience, and lies at the heart of defining the biology of cognition and psychiatric disorders. Glutamatergic pyramidal neurons (PyNs) constitute ~80% of cortical neurons, are endowed with large capacity for information coding, storage, and plasticity, and carry the output of cortical computation. PyNs consist of diverse subtypes based on their specific laminar locations, axonal projection patterns, and gene expression profiles. Subsets of PyNs form multiple and hierarchical subnetworks of information processing, with distinct output channels to cortical and subcortical targets that subserve sensory, motor, cognitive, and emotional functions. Importantly, PyN subtypes are differentially affected in various neuropsychiatric and neurodegenerative disorders. However, the serious lack of specific and effective genetic tools for studying PyNs has hampered progress in understanding cortical circuits. We have begun to build a comprehensive genetic tool set for major PyN subtypes in the mouse through a joint project led by my laboratory and Dr. Paola Arlotta at Harvard University, with key collaboration from Dr. Hongkui Zeng at the Allen Institute for Brain Science. We have discovered a set of specific and combinatorial markers that distinguish major PyN subtypes. We are using intersection, subtraction, and inducible strategies to target PyN subtypes.

The Progenitor Origin of Chandelier Cells

Diverse GABAergic interneurons regulate the functional organization of cortical circuits and derive from multiple embryonic sources. It remains unclear to what extent embryonic origin influences interneuron specification and cortical integration due to difficulties in tracking defined cell types. Using genetic fate mapping, we followed the developmental trajectory of chandelier cells (ChCs), the most distinct interneurons that innervate the axon initial segment of pyramidal neurons and control action potential initiation. A single ChC innervates hundreds of pyramidal neurons and might exert decisive influence on their firing, but the developmental origin and cortical organization of ChCs have been unclear. We recently discovered that the specification of ChC identity is mediated through lineage and birth timing mechanisms in

the embryonic subpallium, and young ChCs navigate over long distances with stereotyped route and schedule to achieve characteristic laminar and areal distribution in the cortex by the end of first postnatal week. The developmental specification of this bona fide interneuron type likely contributes to the assembly of a cortical circuit motif. We have been using combinatorial genetic fate mapping to explore whether there are designated ChC progenitor pools that give rise to this distinct cell type.

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

Epigenetic regulation at 5-methylcytosine (5mC) in the mammalian genome is essential for numerous biological processes, including brain development, function, and plasticity. A key obstacle in genomic and epigenomic analysis of 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 developed an integrated genetic targeting and molecular tagging system that establishes "genetic access" to the methylomes and transcriptomes of specific cell types. This is achieved by cell-type-specific expression of an epitope-tagged histone protein (H2B-GFP fusion protein) through mouse engineering. Using fluorescence-assisted cell sorting (FACS) of dissociated cortices and whole-genome bisulfite sequencing, we have analyzed the DNA methylomes of two cardinal neuron types, the glutamatergic and GABAergic neurons. In collaboration with the Michael Wigler lab, we achieved 10× whole-genome coverage per cell type, allowing us to interrogate ~90% of the CpG sites in the mouse genome. These results identify extensive genome-wide methylation differences in two cardinal neuron types in the postnatal cortex. Furthermore, we have demonstrated, to our knowledge for the first time, developmental dynamics in methylation in a defined cell type during cortical maturation.

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.

MeCP2 Regulates the Maturation of GABA Signaling and Critical Period Plasticity That Shape Experience-Dependent Functional Connectivity in the Primary Visual Cortex

A major challenge is to explain the cellular, developmental, and neural circuitry mechanisms that link gene mutations to functional and behavioral deficits. Rett syndrome (RTT) in several ways epitomizes this challenge: RTT is caused by mutations in the *MeCP2* gene that encodes a broadly expressed chromatin and transcription regulatory protein, and yet it is characterized by postnatal language and cognitive deficits, stereotyped behaviors, and a myriad of sensory and neurological symptoms. Although numerous mouse models of RTT have been established, it has been difficult to identify primary molecular and cellular changes and trace their impact on circuit alterations that underlie behavioral deficits. Using *MeCP2-null* mice, we examined experience-dependent development of neural circuits in the primary visual cortex where GABAergic interneurons regulate a critical period of plasticity. The functional maturation of parvalbumin interneurons was accelerated upon vision onset, as indicated by the elevated GABA synthetic enzyme GAD67 and perineuronal net formation. These are correlated with an early enhancement of GABA transmission within the parvalbumin interneuron network and an accelerated maturation of cortical activity propagation patterns. We further demonstrate an early onset and closure of the critical period and deficient visual function: Binocular neurons in mature V1 remain mismatched with regard to inputs from the two eyes. Importantly, a reduction of GAD67 level is sufficient to rescue precocious onset of the critical period, suggesting a causal role for GAD67 in MECP2-mediated regulation of experience-driven V1 circuit development. Taken together, our study establishes a coherent link, to our knowledge for the first time, from specific molecular changes in defined cell types to circuit development and plasticity, and to a functional alteration with behavior implications, in a mouse model of RTT. Our findings thus begin to elucidate the circuit pathogenesis by "connecting the dots" across levels from neural development to function.

Input-Specific Maturation of Synaptic Recruitment of Parvalbumin Interneurons in the Primary Visual Cortex

Cortical networks consist of local recurrent circuits and long-range pathways from other brain areas. Parvalbumin inhibitory interneurons (PVNs) regulate the dynamic operation of local ensembles as well as the temporal precision of afferent signals. The synaptic recruitment of PVNs that support these circuit operations is not well understood. Here, we demonstrate that the synaptic dynamics of PVN recruitment in mouse visual cortex are customized according to input source with distinct maturation profiles. Although the long-range inputs to PVNs show strong short-term depression throughout postnatal maturation, local inputs from nearby pyramidal neurons progressively lose such depression. This enhanced local recruitment depends on PVN-mediated reciprocal inhibition and results from both pre- and postsynaptic mechanisms, including calcium-permeable AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors at PVN postsynaptic sites. Whereas short-term depression of long-range inputs is well-suited for afferent signal detection, the robust dynamics of local inputs may facilitate rapid and proportional PVN recruitment in regulating local circuit operations.

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NEUROBIOLOGY OF COGNITION AND DECISION-MAKING

A. Kepecs L. Calcaterra J. Hirokawa H.J. Pi
E. Demir D. Kvitsiani S. Ranade
L. Desban M. Lorenc J. Sanders
B. Hangya P. Masset A. Vaughan

Understanding the mysteries of cognition has been an age-old aspiration of humankind. Our laboratory engages this question by studying the neurobiological basis of cognitive tasks in rodents. We use a reductionist approach to translate psychological questions into the language of neuroscience by developing quantifiable, well-controlled behavioral tasks for rodents. We then couple these tasks with targeted, high-resolution, and high-throughput monitoring and manipulation of neural circuits mediating cognitive behaviors. Given the complexity of animal behavior and the dynamics of neural networks producing it, our studies rely on computational models to guide and sharpen the neurobiological questions. Finally, we use human psychophysics to validate our behavioral observations in rodents by linking them with analogous behaviors in humans. Using this integrated approach, our long-term goal is to "reverse engineer" the computational and neurobiological principles underlying cognition and decision-making.

In terms of topics, our approach is multifaceted: We study (1) the roles of uncertainty and confidence in decision-making, (2) foraging decisions about whether to stay or to switch, (3) the division of labor between different cell types in the prefrontal cortex, (4) how the cholinergic system supports sustained attention, and (5) 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 addiction, major depression, schizophrenia, Alzheimer's disease, and autism spectrum disorder.

Neural Basis of Decision Confidence

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

Natural events in the world, and the likely consequences of our actions, are both fraught with ambiguity. In the face of pervasive uncertainty, one's degree of confidence in a belief is a critical component of cognition. This can confer benefits for a broad range of activities from the sophisticated to the mundane to the essential: managing a stock portfolio, deciding whether to carry an umbrella, or deciding between fight and flight. An essential use of confidence estimates is to guide information-seeking behaviors, learning, and attention so as to reduce the level of uncertainty. Conversely, the pathological misevaluation of confidence contributes to a wide range of neuropsychiatric conditions, including anxiety, obsessive-compulsive disorder, and addiction.

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 basis 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 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 will 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.

From Metacognition to Statistics: Confidence Judgments in Humans

J. Sanders, B. Hangya, P. Masset

In our rodent studies of confidence, we use a computational framework to interpret behavioral and neural data. We are also interested in understanding the degree to which the theoretical concept of *decision confidence* corresponds to the human notion of subjective confidence. Human confidence judgments are thought to originate from metacognitive processes that provide a subjective assessment about one's beliefs. Confidence can be alternatively framed as an objective statistical quantity, an estimate of the probability that a chosen hypothesis was correct. Despite similar usage of this term, it remains unclear whether the objective, statistical notion of confidence is related to the subjective human feeling of confidence.

To address this issue, we developed a normative statistical framework to mathematically derive predictions relating a statistical measure of decision confidence to observable measures of decisions. We found that human confidence reports in both sensory and general knowledge decision tasks followed the normative statistical predictions, defying several naïve intuitions about confidence. In addition, by fitting a model to choice behavior within this framework, we could quantitatively predict confidence reports. On the basis of these results, we suggest that the computation underlying the human sense of confidence is consistent with a mental implementation of statistical decision confidence.

Next, we developed 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. We designed a perceptual discrimination task where subjects choose which of two Poisson click streams is clicking faster and rate their confidence in their choices. On each trial, we store the precise time of each click the subject heard in each ear and acquire three measures: a reaction time (how long the subject collected evidence before choosing), a choice (which side they thought was faster), and a confidence report (how much they believe in the hypothesis that their choice was correct). We used several previously described models of choice and confidence to try to design a computer program that could "listen" to the same click trains and respond with the same reaction time, choice, and confidence. To our surprise, we discovered that standard models of perceptual decision-making failed to reproduce the basic patterns from our normative model, that confidence reflects the strength of perceived evidence. Currently, we are examining a broader range of models to fit our psychophysical data.

We find that all of these confidence-reporting tasks share the basic patterns of confidence that is predicted by statistical confidence. On the basis of these results, we are now in a position to use these quantitative measures of decision confidence in humans, through collaborations and 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.

The Representational Content of Orbitofrontal Cortex during Outcome Anticipation

J. Hirokawa, A. Vaughan

OFC is strongly implicated in decision-making under uncertainty, especially when decisions require the evaluation of predicted outcomes. Neurophysiological studies conceptualize OFC as a hub for the integration of different variables into unified value signals. Using model-based approaches, previous studies have identified a number of critical outcome-related variables in OFC, such as reward value, risk, and confidence. However, such model-based approaches are limited in their ability to characterize neuronal representations

because it is difficult to address all potentially encoded variables. Here, we address the representational content of OFC using both model-based and model-free (i.e., unsupervised) approaches. First, we assessed whether and how different decision variables are integrated in OFC using a model-based approach. Second, we sought to uncover the content of OFC representations using unsupervised techniques to assess whether decision variables arise naturally and whether they are represented separately or as a continuum.

To address this, we trained rats in a two-alternative forced-choice (2AFC) perceptual decision-making task with block-wise changes in reward size and recorded 481 neurons in ventrolateral OFC. We focused our analysis on the period after choice/before feedback (i.e., while the animal is anticipating the outcome of its choice). First, we constructed a normative model to account for the behavioral data. Our model combines reward value and perceptual decision confidence in a Bayesian way to yield integrated value, which is then used to make choices. The model correctly predicted the behavioral choices and reaction times observed in each animal. From neuronal data, analysis of single units revealed that individual OFC neurons can represent identifiable decision variables separately, including reward size and confidence, as well as their combination into integrated reward value. Analysis across the population of OFC neurons revealed the separable representation of a variety of decision variables predicted by the model. Importantly, the largest fraction of neuronal variance in the OFC population (the first principal component) was explained by a variable, which represents coherently integrated value (decision confidence multiplied by reward size). Moreover, cluster analysis suggests nonrandom, structured representations of these decision variables in OFC. Taken together, we demonstrated that OFC representations of task variables are fundamentally linked to model-based decision variables such as reward value and decision confidence.

Microtraits in Rodent Models of Psychiatric Diseases

L. Desban, J. Hirokawa [in collaboration with F. Henn, Cold Spring Harbor Laboratory]

Basic research on psychiatric disorders relies on the use of animal models. Usually, animal models are validated mainly on the basis of genetic insights and simple behavioral measures. However, in the case of cognitive diseases, this has become challenging because it is unclear how to map human behavioral deficits to animal models. To overcome these issues, we are taking a different approach to this challenge, based on the emerging field of computational psychiatry. We focus on microtraits that can be easily quantified using psychometric tasks and that can be directly related to humans (e.g., reward sensitivity, learning rate, and decision confidence).

We attempted to use such a dimensional approach to identify microtraits that may be quantitatively different between our model rats and their controls. We studied major depressive disorder (MDD), a heterogeneous condition whose pathophysiology remains unclear, with a commonly used rodent model, the congenital learned-helplessness (cLH) rat. Using different behavioral paradigms, we tested two major hypotheses about depression: anhedonia, a blunted sensitivity to reinforcers, and depressive realism, the improved calibration of the sense of confidence. We first manipulated reward contingencies by introducing a bias in either reward size or reward probability. Our results showed no significant difference between cLH and cNLH rats, which suggested that cLH rats did not display any imbalance in reward evaluation. Then, we assessed confidence based on their willingness to invest waiting time for uncertain outcomes. When they were most confident, the optimal strategy was to wait longer, and indeed, this is what we observed. Moreover, we observed greater differences in cLHs' waiting times with confidence, suggesting that they had more insight into their choices than controls during the task. These preliminary results highlight how a computational phenotyping approach can be used to relate animal models of psychopathologies to human behavior.

Impact of Drugs of Abuse on Decision-Making and Orbitofrontal Cortex

Y. Wu, J. Hirokawa

Drug abuse and addiction result in and are compounded by compromised decision-making processes. OFC has emerged as one important region of structural and functional alterations resulting from chronic drug exposure. Although opioids are widely

used for treatment of chronic pain conditions, their long-term effects on cognitive functions have not been well characterized. Morphine is a potent opiate drug that has been shown to directly affect OFC, along with other prefrontal regions with strong dopaminergic projections. Addiction to morphine renders subjects unable to make good judgments and flexible choices, which mimics the effects of OFC lesions. Therefore, we sought to find out whether the previously unexplained behavioral aspects of drug addiction could be due to defects in integration of decision confidence and reward value in the decision-making process.

In our preliminary experiments, we used daily sessions of morphine injections to disrupt choice behavior or saline controls. We found that acute effects of morphine involve a decrease in choice accuracy and increase in impulsivity and reaction time. In addition, although saline-treated rats show a strong tendency to pick a bigger reward when the evidence level is low, this tendency is decreased in morphine-treated rats, and the severity of this tendency is correlated with the amount of morphine animals received. These results are consistent with morphine causing defects in the integration of reward value and sensory evidence. On the basis of this evidence, we are pursuing the hypothesis that morphine self-administration leads to suboptimal decision-making by disrupting integration and distribution of decision variables in OFC.

Foraging Decisions, Anterior Cingulate Cortex, and Inhibitory Neuron Types

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

Making decisions about when to engage with options as they are encountered and when to disengage and switch behavior are fundamental problems for all decision makers. Such decisions about whether to stay and exploit an opportunity or leave and explore alternatives, presented in the language of behavioral ecology, are called foraging decisions. It is critical to appreciate that foraging decisions are not binary ones between currently available options (such as two-alternative forced choice), but are instead decisions about when and how to engage with the available options.

We have been studying the anterior cingulate cortex (ACC) during a simple foraging task. In both humans and rodents, ACC has been implicated in variety of goal-directed behaviors including reward processing, inhibitory response control, and conflict monitoring. Electrophysiological recordings from ACC show great diversity of neuronal responses to a range of behavioral variables. At the same time, the neocortex is composed of a variety of cell types, among which GABAergic interneurons exhibit the largest 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 used optogenetics as a means of identifying extracellularly recorded neurons in freely moving mice, focusing on inhibitory interneurons, which exhibit the largest diversity of cell types in cortex. We tested the overarching hypothesis that neurons belonging to the same subtype share fundamental commonalities in response properties during behavior, whereas distinct subtypes specialize in distinct functional roles.

We showed that parvalbumin-expressing (Pv) and a subtype of somatostatin-expressing (Som) neurons form functionally homogeneous populations showing a double dissociation between both their inhibitory impact and their behavioral correlates. Out of a number of events pertaining to behavior, a subtype of Som neurons selectively responded at reward approach, whereas Pv neurons responded at reward leaving, encoding preceding stay duration. These behavioral correlates of Pv and Som neurons defined a behavioral epoch and a decision variable important for foraging (whether to stay or to leave), a crucial function attributed to ACC. Furthermore, Pv neurons could fire in millisecond synchrony, exerting fast and powerful inhibition on principal cell firing, whereas the inhibitory impact of Som neurons on firing output was weak and more variable, consistent with the idea that they respectively control the outputs of and inputs to principal neurons. These results suggest a connection between the circuit-level function of different interneuron types in regulating the flow of information and the behavioral functions served by the cortical circuits. Our results also point to a new view of inhibition that sees interneurons as encoding behaviorally relevant variables and serving to control the flow of information on behavioral timescales.

Cortical VIP Interneurons and Disinhibitory Control

H.J. Pi, B. Hangya [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 direct perception and behavior. As part of this broader project, we studied vasoactive intestinal peptide-expressing (VIP) interneurons. VIP neurons are mostly bipolar, and their processes are vertically oriented and constitute less than 2% of all cortical neurons. Interestingly, these neurons specifically target other inhibitory neurons and avoid pyramidal cells. Therefore, we hypothesized that VIP neurons regulate a disinhibitory circuit and tested it in two functionally different cortical regions: auditory and medial prefrontal cortices.

Using a combination of in vivo and in vitro physiology, we showed that VIP interneurons mediate disinhibitory control in multiple areas of neocortex. By combining optogenetic activation with single-cell recordings, we examined the functional role of VIP interneurons in awake mice and investigated the underlying circuit mechanisms in vitro in auditory and medial prefrontal cortices. We identified a basic disinhibitory circuit module in which activation of VIP interneurons transiently suppresses most somatostatin-expressing and a fraction of parvalbumin-expressing inhibitory interneurons—specialized to control the input and output of principal cells, respectively. In the auditory cortex, we found that reinforcement signals (punishment and reward) strongly activated VIP neurons, and in turn, VIP recruitment modulated the tone-responsiveness of a functional subpopulation of principal neurons. We propose that this disynaptic disinhibitory mechanism represents a cortical circuit motif that enables long-range inputs to modulate the gain of local circuits.

What are the mechanisms by which VIP neurons are recruited at specific moments in behavior? Previous studies suggest that VIP neurons may act as a conduit for fast neuromodulatory control and thereby acquire behaviorally relevant responses. To identify which neuromodulatory systems recruit a VIP-controlled disinhibitory circuit, we took advantage of a rabies-virus-assisted retrograde tracing system that only labels monosynaptic input neurons fluorescently. We identified direct projections from cholinergic neurons in the basal forebrain and serotonergic neurons in the raphe nuclei. Moreover, it is known that VIP neurons express ionotropic nicotinic acetylcholine and serotonergic 5-HT_{3A} receptors. On the basis of these results, we are pursuing the hypothesis that VIP neurons transform fast neuromodulatory signals into cortical disinhibitory output.

Neural Representation of Social Decisions and Rewards

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

Social behavior is integral to animals' survival and reproduction. Social deficits are at the heart of cognitive disorders such as autism spectrum disorder that have proven to be profoundly difficult to study in model organisms. 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 started our studies by recording from part of the amygdala that processes information from the vomeronasal system, detecting behaviorally significant chemical cues such as pheromones. We have designed a behavioral task that presents different urinary proteins to our mice while we are performing electrophysiology in medial amygdala. Moreover, we characterized molecular markers that enable us to further dissect the social amygdalar circuit. Currently, we are doing optogenetic experiments as well as electrophysiological recordings to map behavioral relevance onto specific medial amygdala neurons.

We are also interested in understanding the basic rules that mice use to choose their partners. For this purpose, we have developed a psychophysical social behavior task, the "social carousel," inspired by perceptual psychophysics and game theoretic traditions that have been instrumental in studying other facets of cognition. Our task enables the reliable, quantitative, and high-throughput analysis of social interactions in

mice. This task contrasts appetitive rewards with opportunities for social interaction. Water-deprived animals are trained to alternate between two platforms: One platform provides a small water reward and the other allows for social interaction with caged mice drawn at random from a revolving magazine. Subjects can engage in extended social interactions with these mice, but at the expense of delaying the water reward. This task allows us to infer the "social value" of a mouse based on the trade-off between social interactions and appetitive rewards. In addition, this task is compatible with our electrophysiological studies because it is devised for precise stimulus delivery and reproducible behavioral contingencies. Currently, we are characterizing social preferences of individual mice of different strains and subspecies.

Role of Nucleus Basalis in Reinforcement and Sustained Attention

B. Hangya, S. Ranade, M. Lorenc

The nucleus basalis (NB) is a vitally important yet poorly understood neuromodulatory system that is thought to have significant roles in cognitive functions, including learning, memory, and attention. Cognitive deficits in Alzheimer's disease, Parkinson's dementia, age-related dementias, and normal aging are correlated with the extent of deterioration of NB cholinergic neurons. Yet, despite the association of NB with higher cognitive functions and a host of disease states in humans, it is surprising how little we understand about its function during behavior. Previous research, mostly using lesions, pharmacology, and microdialysis, revealed that NB can have strong and confusingly diverse effects on downstream targets and behavior. However, it is not known when cholinergic neurons are recruited during behavior and how their activity might support different aspects of cognition.

To overcome these challenges, we combine optogenetic and electrophysiological approaches to record from identified cholinergic projection neurons in NB during behavior. We have successfully developed visual and auditory versions of sustained attention tasks for mice. In these tasks, mice report target stimuli occurring at uncertain times. Performance and reaction time are used as indicators of attention. We manipulate temporal expectancy by using specific distributions for stimulus timing, thus modulating attention in a temporally precise, graded manner, also reflected in the reaction times of the animals. We recorded single-neuron activity from the NB while mice were performing the auditory sustained attention task and identified cholinergic neurons by using an optogenetic approach. We found that tonic changes in cholinergic firing are correlated with brain states but not with behavioral measures of attention on a trial-to-trial basis. On the other hand, cholinergic neurons were phasically activated following delivery of the reinforcer, either air puff or water reward. Surprisingly, almost all cholinergic neurons recorded during the task showed a similar fast response to the air puff with a median latency of 17.5 msec and a very low jitter (median jitter = 2.2 msec), whereas responses to reward were more variable. These results suggest that cholinergic neurons have dual functions, with tonic firing mediating vigilance and phasic firing rapidly signaling reinforcement feedback.

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THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

A. Koulakov D. Ferrante Y. Wei

Our laboratory develops theories of neural computation. We work in parallel on three topics. First, we formulate mathematical models for combining genetic information and experience (nature and nurture) during formation of connections between neurons. Our models describe 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. These models have been tested on circuits that are formed in the visual system and can be rewired through genetic, surgical, and pharmacological manipulations. We have applied our theory to the visual maps of ocular dominance observed in visual cortices of many higher vertebrates, such as humans. We were able to relate the orientation of ocular dominance patterns to the distribution of molecular labels that control establishing connectivity between the cortex and thalamus. Second, we have been developing the neural network theory for olfactory processing. We previously argued that information about smells can be represented in the olfactory system in the form of temporal sequences. We proposed a model for learning odorant representations in the olfactory bulb. Finally, this year, we have developed a model for decision confidence estimation within cortical networks forming perceptual decisions.

The Molecular Basis for the Development of Neural Maps

Y. Wei, A.A. Koulakov

Neural development leads to the establishment of precise connectivity in the nervous system. By contrasting the information capacities of cortical connectivity and the genome, we suggest that simplifying rules are necessary in order to create cortical connections from the limited set of instructions contained in the genome. One of these rules may be used by the visual system, where connections are formed on the basis of the interplay of molecular gradients and activity-dependent synaptic plasticity. We show how a simple model that accounts for such interplay can create both neural topographic maps and more complex patterns of ocular dominance (i.e., the segregated binary mixture of projections from two eyes converging in the same visual area). With regard to the ocular dominance patterns, we show that pattern orientation may be instructed by the direction of the gradients of molecular labels. We also show that the periodicity of ocular dominance patterns may result from the interplay of the effects of molecular gradients and correlated neural activity. Overall, we propose that simple mechanisms can account for the formation of apparently complex features of neuronal connections (Fig. 1).

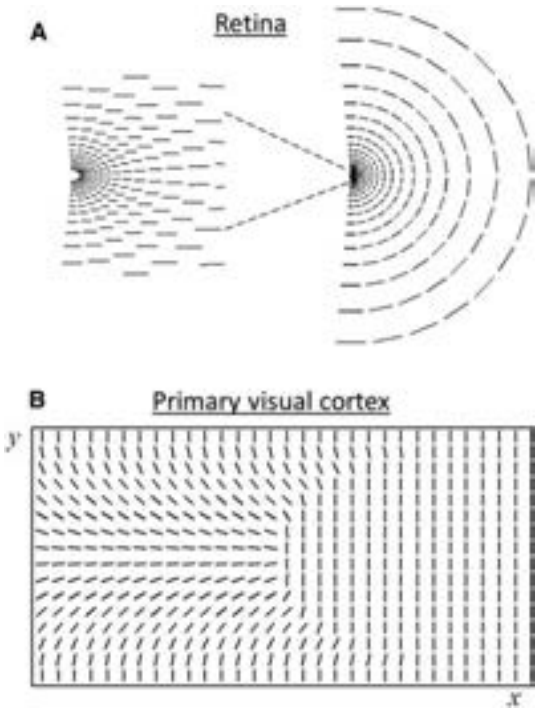

Figure 1. The geometry of the ocular dominance stripe orientation predicted by our computational model. (*A*) The orientation of ocular dominance stripes in the middle of the eye is predicted to be horizontal, whereas at the periphery, this orientation is circular. (*B*) This is a picture of orientation projected into the primary visual cortex.

Sparse Learning of Odorant Representations in the Olfactory Bulb

A. Koulakov [in collaboration with S. Shea, Cold Spring Harbor Laboratory]

The olfactory bulb is the first brain region in the neural network dedicated to odor perception. Mitral cells in the olfactory bulb receive odor information from nasal epithelial receptor neurons and process and transmit this information to the cortex. Activity at this crucial processing step is steeply dependent on behavioral state and experience. For example, sustained mitral cell odor responses under anesthesia disappear in wakefulness. Yet, aligning spikes to respiration revealed brief and precise transient activity, suggesting that odor information is transmitted as sparse temporal sequences of activation. Mitral cell output is likely shaped in part by local inhibitory neurons called granule cells, but the significance of granule cells for sensory coding and perception is unclear. Recently, we proposed that granule cells form inverse representations of odorants that are subtracted from the responses of mitral cells to eliminate redundancies and enforce temporal structure. The responses of cells in the olfactory bulb circuit display rich dynamical repertoire including state-dependent activity, temporal coding, and experience-dependent plasticity. Here, we extended our theoretical model and derived the learning rules for granule cell connections that facilitate sparse, temporally patterned mitral cell output that reduce the redundancy of odorant representations by the mitral cells. In parallel, we used experimental data to rigorously test our model's predictions. Understanding how redundancy gets reduced in the mitral-granule cell network is especially relevant for the human olfactory system because according to recent data, it contains a greater number of mitral cells receiving redundant inputs from fewer types of olfactory receptor neurons. Moreover, sparsened sensory coding is observed in a wide range of systems during wakefulness. Our studies help us to understand how this computation is achieved by experience-dependent plasticity and learning in a population of olfactory inhibitory neurons.

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THE FUNCTION AND PLASTICITY OF CENTRAL SYNAPSES IN ADAPTIVE AND MALADAPTIVE BEHAVIORS RELATED TO PSYCHIATRIC DISORDERS

B. Li S. Ahrens G.-R. Hwang M. Stephenson-Jones
K. Delevich M. Penzo K. Yu

Understanding the relationship among synapse, circuit, and behavior has been the focus of research in my lab. We are particularly interested in understanding the synaptic and circuit mechanisms underlying cognitive functions, as well as synaptic and circuit dysfunction that may underlie mental disorders, including anxiety, depression, schizophrenia, and autism. To address these questions, we use in vitro and in vivo electrophysiology, two-photon imaging, and molecular, genetic, optogenetic, and chemical-genetic methodologies to probe and manipulate the function of specific neural circuits in the rodent brain and to determine their role in behaviors, such as learning and expression of fear, learned helplessness, and attention. We are currently undertaking three major lines of research, which are summarized below.

The Central Amygdala Fear Circuits and Fear Regulation

The amygdala is essential for fear learning and expression. The central amygdala (CeA), once viewed as a passive relay between the amygdala complex and downstream fear effectors, has emerged as an active participant in fear conditioning. However, how CeA contributes to the learning and expression of fear is unclear. We recently discovered in mice that fear conditioning induces robust plasticity of excitatory synapses onto inhibitory neurons in the lateral subdivision of CeA (CeL). This experience-dependent plasticity is cell-specific and expressed by inputs from the lateral amygdala. Furthermore, we demonstrated that this CeL plasticity is required for the storage of fear memories.

The CeL also controls fear expression by gating the activity of the medial subdivision of the central amygdala (CeM), the canonical amygdala output to areas that mediate defensive responses. In addition to the connection with CeM, the CeL sends long-range projections to innervate extra-amygdala areas. However, the long-range projection CeL neurons have not been well-characterized, and their role in fear regulation is unknown. Our recent studies in mice show that a subset of CeL neurons directly project to the midbrain periaqueductal gray (PAG) and the paraventricular nucleus of the thalamus, two brain areas implicated in defensive behavior. These long-range projection CeL neurons are predominantly somatostatin-positive (SOM^+) neurons, which can directly inhibit PAG neurons, and some of which innervate both the PAG and the paraventricular nucleus of the thalamus. Notably, fear conditioning potentiates excitatory synaptic transmission onto these long-range projection CeL neurons. Thus, our study identifies a subpopulation of SOM^+ CeL neurons that may contribute to fear learning and regulate fear expression independent of CeM.

The Habenula Circuitry in the Learned Helplessness Model of 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. We have found that in two learned helplessness models of depression, 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 for patients who are depressed, markedly 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.

This study provides insights into cellular mechanisms that may explain previously reported phenomena—the increase in LHb metabolic activity observed in humans who are depressed and in animal models of depression—and that lesion or pharmacological silencing of the LHb can modulate depression-like symptoms in animal models. Our findings suggest an aberrant cellular process that has not previously been examined in the context of mood disorders and that may be crucial in the etiology of depression. Future studies aimed at determining the changes in molecular signaling that underlie the synaptic hyperactivity onto LHb neurons may lead to novel and effective treatments able to reverse some forms of depressive disorders.

The Schizophrenia-Linked ErbB4 Controls Attention through the Thalamic Reticular Nucleus

Considerable evidence supports a role of the thalamic reticular nucleus (TRN) in sensory processing and cognitive functions such as attention. Dysfunction of TRN has been implicated in schizophrenia, a mental disorder in which cognitive deficit is a core feature; however, the mechanisms by which TRN dysfunction occurs and leads to disease symptoms are unclear. We have probed and manipulated the somatostatin (SOM^+) class of TRN neurons, which express *ErbB4*, a gene that has been linked to schizophrenia. We found that *ErbB4* deficiency in these neurons selectively strengthened excitatory synapses driven by cortical inputs, thereby enhancing the feedforward modulation of thalamic neurons, and specifically impaired behavioral performance in a task that engages rule selection but not input selection, attentional processes dependent on activity of SOM^+ TRN neurons.

Rule selection and input selection represent distinct attentional processes that likely require the coordinated action of cortical and subcortical structures. We found that suppression of SOM^+ TRN neurons (by the chemical-genetic method) impaired performance in both the input-selection and rule-selection tasks, indicating that these neurons are indispensable. On the other hand, increasing cortical drive onto SOM^+ TRN neurons (caused by ErbB4 deficiency) markedly improved input selection, but severely impaired rule selection. These results, although paradoxical at first glance, are in fact consistent with Francis Crick's attentional searchlight hypothesis, in which TRN neurons act as the "beam" of the searchlight to enhance the activity of thalamocortical neurons. Indeed, activation of TRN neurons promotes the generation of bursting activity in the thalamus, which may facilitate information processing and improve behavioral performance. Through this mechanism, the increased cortical drive onto TRN neurons may improve performance in the input-selection task, in which distractors may interfere with information processing in the thalamus through a bottom-up process. In contrast, in the rule-selection tasks, both auditory and visual stimuli have been learned to be associated with reward, and therefore, either stimulus can be voluntarily attended through a top-down process. In a given trial, attending to the inappropriate stimulus (under the current rule) results in performance error. This problem becomes exaggerated in the ErbB4 mutant mice, in which the aberrantly enhanced cortical-TRN inputs might render the TRN neurons more responsive to false signals originating from the cortex.

These results uncover a critical role of a cortico–TRN synaptic circuitry in goal-directed attention and provide insights on cellular and circuit mechanisms by which TRN dysfunction may occur and contribute to a consistently observed cognitive impairment in major psychiatric disorders. Our results suggest that either aberrantly increased or decreased TRN neuronal activity can result in impairment in rule selection, a key aspect of executive control. The rule-selection task used in our study mimics, to some extent, the Stroop task, a test commonly used to assess cognitive deficit in schizophrenia. The finding that ErbB4 deficiency selectively impairs rule selection, but not input selection, parallels a clinical observation that schizophrenia specifically affects performance in the Stroop task and does not impair attentional functions in general.

Deletion of a single copy of *ErbB4* is sufficient to produce a robust effect, suggesting that ErbB4 signaling is exquisitely regulated in SOM^+ TRN neurons in order to properly maintain TRN function. Thus, our findings reveal circuitry and cellular mechanisms that may underlie a form of cognitive deficit observed in mental disorders.

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INTEGRATIVE SYSTEMS NEUROBIOLOGY

P. Mitra N. Baltera, C. Bergano, A. Field-Pollatou, N. Franciotti, P. Grange, H. Greene, G. Havkin, J. Jones, F. Mechler, S. Michelsen, A. Mukherjee, V. Pinskiy, C. Powell, S. Savoia, A. Tolpygo, K. Weber, C. Wu, Y. Zhang

We study complex neurobiological systems using a combination of experimental and computational approaches. The primary area of experimental work in our laboratory is the Mouse Brain Architecture (MBA) project, with a goal to generate a brainwide mesoscale connectivity map in the mouse. In collaborative studies, we also apply the methods developed for the MBA project to multiple other species, including the zebra finch, marmoset, macaque monkey, and human. Our neuroinformatics research involves the development and application of analytical tools to large volumes of neurobiological data. A specific area where we have concerted our efforts is the development of informatics infrastructures for data and knowledge integration for various brain connectivity projects. We have major ongoing collaborations with research groups, both inside and outside CSHL. At CSHL, we collaborate with Josh Huang on a project on GABAergic interneuron expression in mouse models of autism. The Brain Architecture Project has collaborators at multiple institutions, including Harvey Karten (University of California, San Diego), Michael Hawrylycz (Allen Institute of Brain Research), Jin Hyung Lee (Stanford), and Mihail Bota (University of Southern California). We began a joint effort with Allan Johnson's team (Duke University) to develop a high-resolution Nissl atlas for the MR-based Waxholm space of the mouse brain. We collaborate with Bijan Pesaran (New York University) on mapping connectivity in the macaque brain, and with Naoshige Uchida (Harvard) on mapping the afferent inputs to the dopaminergic system of the mouse brain. We have completed the pilot phase of a collaborative effort with Kirk Wilhelmsen (University of North Carolina) to link brainwide neuroanatomy with genetics using the Collaborative Cross project mouse lines. We have also been working with Randal Burns and his team (Johns Hopkins University) on integrated hardware/software solutions to storage and computing tasks posed by the massive imaging data in our projects.

Continuing in the Mitra laboratory in 2014 are Sandra Michelsen (Administrator), Vadim Pinskiy (Computational Science Analyst), Alex Tolpygo (Laboratory Manager), Gregor Havkin (Computational Science Manager), Kevin Weber (Laboratory Technician), Neil Franciotti (Laboratory Technician), Amit Mukherjee (Postdoctoral Fellow), and Ferenc Mechler (Computational Science Analyst, Project Manager). Jamie Jones (Laboratory Technician), Caitlin Powell (Laboratory Technician), Christin Bergano (Laboratory Technician), Harrison Greene (Laboratory Technician), Pascal Grange (Postdoctoral Fellow), Caizhi Wu (Research Associate), and Yingbin Zhang (Computational Science Manager) have recently left to further pursue their careers. Angeliki Field-Pollatou (Visiting Graduate Student), Nicholas Baltera (Laboratory Technician), and Stephen Savoia (Laboratory Technician) joined us during the last year.

Mouse Brain Architecture Project

V. Pinskiy, A. Tolpygo, C. Wu, J. Jones, K. Weber, C. Bergano, N. Franciotti, G. Havkin, Y. Zhang, A. Mukherjee, F. Mechler

The function of the brain is served and constrained by its wiring and our knowledge of a comprehensive wiring map of the mammalian brain. Our knowledge of the wiring map remains highly incomplete. The MBA project, now running in its fourth year, is designed to address this knowledge gap. We use a pair of anterograde tracing and a pair of retrograde tracing methods and make computer-guided precision injections targeting one of 262+ predetermined sites systematically mapped on a three-dimensional grid that covers the entire brain volume. Using this approach, we map brain connectivity in the adult (p56) mouse (C57BL/6J) in four different ways and align these results to the Allen Brain Atlas.

To date, we have processed and released more than 540 brains for public access on the MBA web portal (http://mouse.brainarchitecture.org/), and more brains are being processed and posted on a continuing basis. Although the whole brain still remains to be targeted with injections, some areas are now well-covered (including the somato-motor system). The Mathers Foundation agreed to provide support to allow the continuation and completion of the MBA project.

Much of the current effort deployed in the MBA project is developing hardware and software tools to cope with challenges posed by the very large (~TB size) image data sets. We need to move, store, and process hundreds of these data sets securely and efficiently. Furthermore, the computationally intensive analyses (such as volume registration) of the MBA brains require specialized software solutions to be feasible on these sets that we address in the Johns Hopkins University collaboration. In addition, we continue to work to improve the portal interface and user experience. We have added new browser capabilities and started to add a video to each brain that animates the coronal image stack as a virtual rostrocaudal fly-through.

Regarding future directions, we started a pilot collaboration with Mihail Bota (University of Southern California) in which he manually annotates the labeled monosynaptic afferent and efferent connections of primary motor cortex (MOp) revealed in tracer-injected MBA project brains. This study aims for a comparison with the BAMS database (that Dr. Bota helps curate) and will help validate project data against literature results and highlight new findings.

Most recently, in collaboration with Professor Guenter Giese at the Max-Planck Institute (Heidelberg), we have started to explore the utility of a novel tissue clearing technology he has recently pioneered. Professor Giese's clearing method, unlike many of his competitors', manages to preserve the fluorescent label with high signal-to-noise ratio and thus presents a feasible and faster alternative to our current technology of mapping whole-brain connectivity. Pilot studies using motor cortical injections produced some good three-dimensional data sets (Fig. 1), and we are looking into ways to incorporate this methodology into our pipeline.

With the use of retrograde tracing methods and the high spatial resolution attained in our data, we expect that the MBA project will complement and significantly refine our knowledge of brain connectivity currently available in the literature or from other projects (in particular, the Allen Institutes' parallel project, which is focused on cell-type-specific projection mapping but only uses anterograde neuronal tracers).

Outreach. We continue to work to highlight the MBA project to the scientific public. I have participated in the U.S. BRAIN initiative related discussions, through online articles and other media listed below:

Scientific American
http://www.scientificamerican.com/article/whats-wrong-with-the-brain-activity-map-proposal/; http://www.scientificamerican.com/article/neuroscientists-weigh-in-obamas-brain-initiative/

The Washington Post
http://www.washingtonpost.com/blogs/wonkblog/wp/2013/04/03/obama-braaaaaains-partha-mitra-whoa-there-buddy/

Live Radio
http://www.kqed.org/a/forum/R201304030900

Published Opinion Papers
Devor et al. (2013); Mitra et al. (2013)

Organizational activity with the National Science Foundation for BRAINI workshops
http://physicsoflivingsystems.org/brainstructureandfunction/

Control Systems Theory Applied to Complex Brain Circuits

P. Mitra

I have continued to pursue theoretical work. I am currently engaged with collaborators Dr. Bassam Bamieh (University of California, Santa Barbara) and Dr. Anirvan Sengupta (Rutgers University) in an investigation that aims to apply novel insights from the theory of distributed control in statistical physics and engineering to biological systems of large numbers of heavily interacting components. The study is motivated by recent discoveries of sharp transitions between states with qualitatively different behaviors that can emerge due to small changes in parameters, analogous to the transitions, for example, between solid and

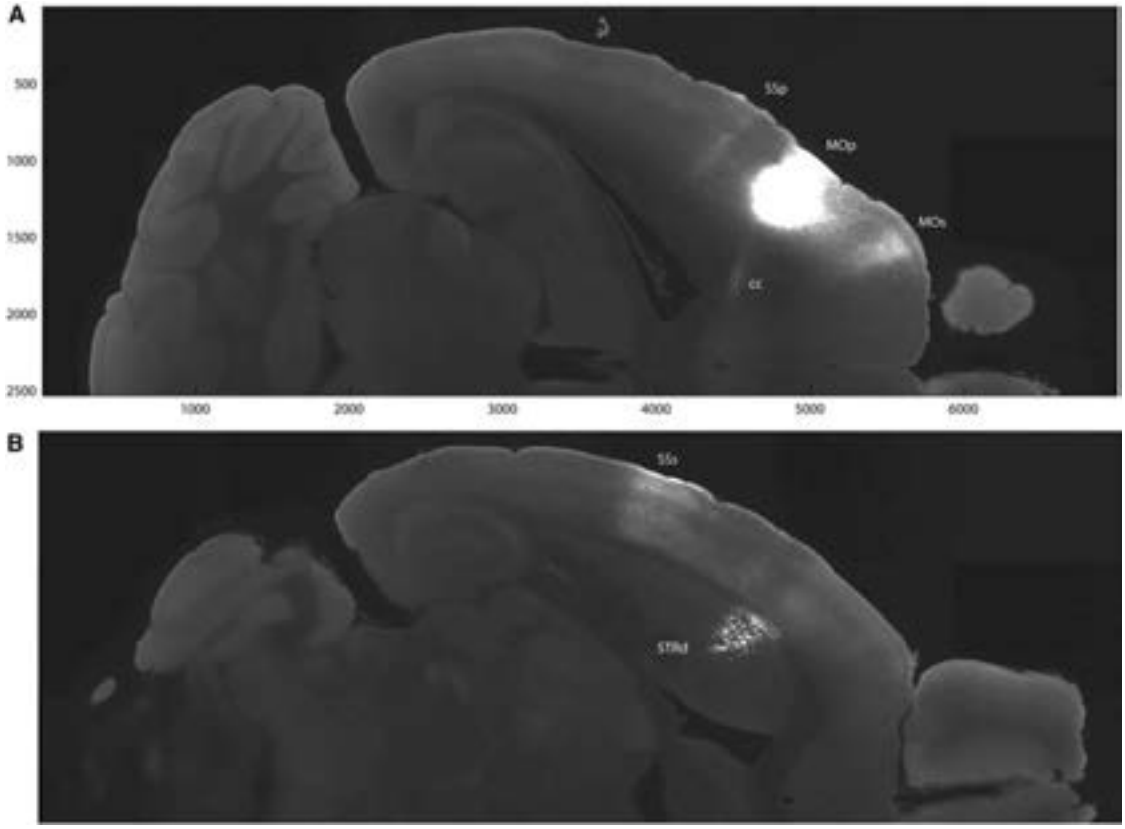

Figure 1. Monosynaptic efferent connections of mouse primary motor cortex (*MOp*) revealed in a mouse brain microinjected in the left MOp with the GFP-expressing anterograde virus tracer, AAV. The whole brain was scanned via light-sheet microscopy (Giese lab), producing a *z*-stack of 3.1-µm-thick optical horizontal slices; here two layers of interest in the left hemisphere are shown in gray level rendition. GFP-labeled axons of AAV-infected MOp neurons make MOp projection fields appear bright. (*A*) *z*-level ~1.8 mm below bregma; (*B*) *z*-level ~2.4 mm below bregma. *MOs*, ipsilateral secondary motor area; *SSp*, *SSs*, ipsilateral primary and secondary somatosensory area; *STRd*, dorsal striatum, descending projection passing through; *cc*, corpus callosum, projections to the contralateral motor cortex.

liquid states. Drawing on National Science Foundation support through the prestigious Inspire Award, we aim to apply these new ideas to help unravel the complex dynamics of the interconnected brain networks. In particular, this novel approach could bring new simplifying insights into the function (and limitations) of the mouse brain from the underpinnings of the connectivity patterns revealed in the MBA project in our lab. For a related groundbreaking theoretical study (Bamieh et al., *IEEE Trans Automat Control 57:* 2235 [2012]), my co-authors and I have received the George S. Axelby Outstanding Paper Award of the Control Systems Society (CSS) of the Institute of Electrical and Electronics Engineers (IEEE). Further information can be found on these theoretical efforts in a recent CSHL press report (http://www.cshl.edu/Article-Mitra/cshls-partha-mitra-receives-two-honors-for-theoretical-work-with-implications-for-brain-circuitry) and links therein.

A High-Resolution Nissl-Based Mouse Brain Reference Atlas

V. Pinskiy, A. Tolpygo, A. Mukherjee, F. Mechler

A classical segmentation of reference atlas brains uses information on cell size and density revealed by Nissl stain. However, the quality and spatial resolution of the Nissl material associated with the available best reference atlases of the mouse brain is limited. To

improve on this, we have begun a collaboration with Professor Allan G. Johnson's team at Duke University to develop a digital high-resolution Nissl-based whole-brain atlas for the adult (p56) C57BL/6J mouse. The Duke team took high-resolution MRI scans (uniform 22-μm voxels) of the brains in the skull shortly after perfusion. The fixated brain-in-skull specimens were processed in the MBA pipeline to acquire high-resolution (0.5 μm/pxl, in-plane) light-microscopic scans of contiguous 10-μm-thick Nissl-stained coronal sections. For each brain, the Nissl image stack was computationally registered to its MR scan and segmented (Fig. 2). A high-quality segmented Nissl brain obtained by averaging several similarly processed mice will update the histological component of the digital Waxholm reference space for multimodal mouse brain images, providing the higher spatial resolution suitable for MBA brains.

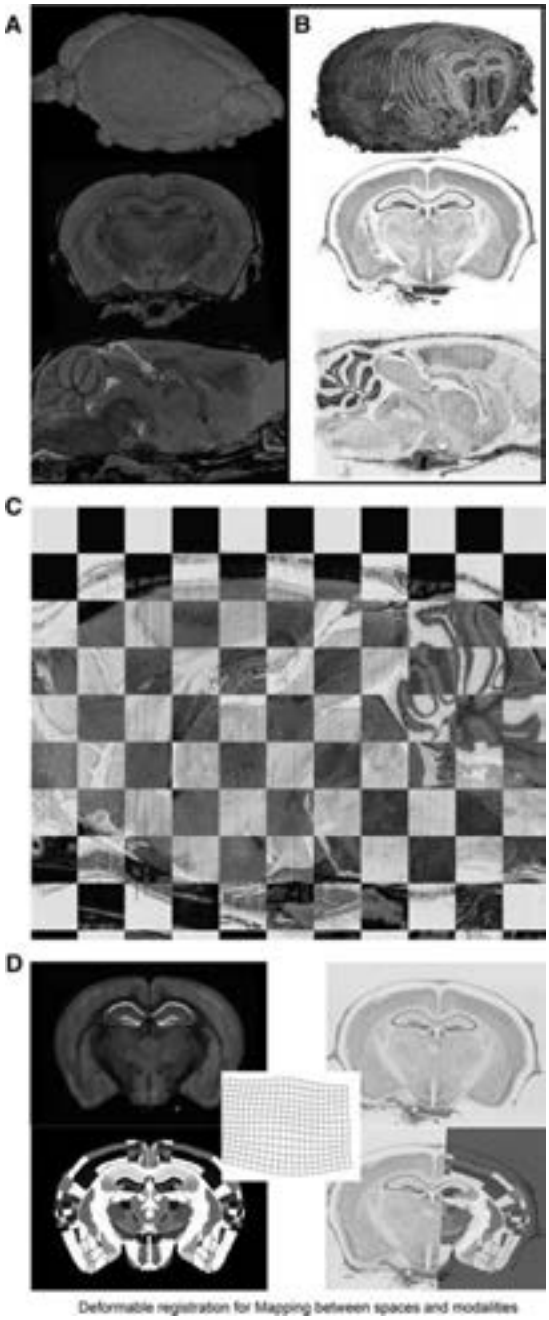

Figure 2. (*A*) The MRI scan of a mouse brain. (*B*) The Nissl stack of the same brain. (*C*) The alignment of the two volumes shown for a sagittal slice through a checkerboard aperture that alternates visibility of the two image modalities to aid visual assessment of their alignment. (*D*) Segmentation of the same Nissl brain is obtained via alignment with the annotated reference brain. (The latter was developed by Dr. Pavel Osten from brains scanned by block-face tomography and registered to the Allen Reference Atlas.) *Inset* is a cartoon of the deformation field of the warping operation that achieves alignment.

Alterations in Brain-Wide GABAergic Neuroanatomy in Autism Mouse Models

V. Pinskiy, A. Tolpygo, A. Mukherjee, F. Mechler

In an ongoing collaboration with Josh Huang, funded by the Simons Foundation Autism Research Initiative (SFARI), we address the developmental neuroanatomy of dysfunctional inhibition implicated in autism spectrum disorders (ASDs). In a systematic brain-wide approach, we generate whole-brain maps of genetically targeted key subpopulations of inhibitory neurons that have distinct physiological function reflected in their specialized cellular morphology. We quantify and compare the distribution and long-range projection patterns of these GABAergic neurons in an ASD mouse model (the *16p*(df/+) heterozygous copy number–deficit mutant) and the "wild type" (C57BL/6). Last year, we reported on baseline data obtained in the wild type; since then, we have bred ASD mutants and started to process them. Figure 3 compares the brainwide distribution of the somatostatin-expressing (SOM+) GABAergic bitufted neurons in one ASD animal and its littermate (wild type) control. Data sets like these are analyzed using software tools we have developed for the MBA computational pipeline. Aligned whole-brain image stacks are registered to a reference atlas, and the normalized cell counts obtained with automatic cell detection within annotated anatomical compartments are compared across genotypes.

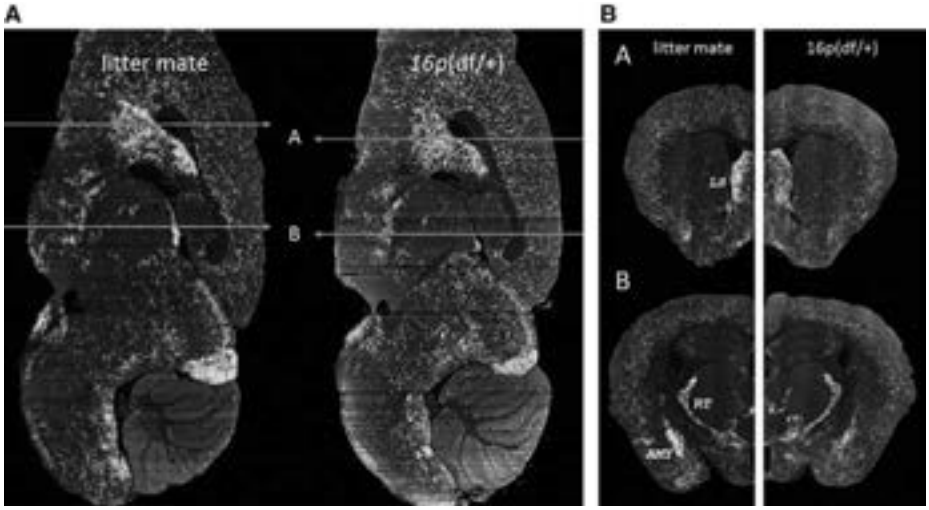

Figure 3. Comparison of the brainwide distribution of genetically targeted somatostatin+ (SOM) GABAergic bitufted neurons in an animal of the ASD model genotype, *16p*(df/+), (*right*) and its wild-type (C57BL/6) littermate (*left*). In these animals, the SOM neurons selectively express nuclear green fluorescent protein by design. The SOM-Cre and HG alleles are heterozygous in both animals. Coronal levels, indicated by arrows in the sagittal sections above, are selected for representative SOM-density patterns: (*A*) ~ Bregma + 1.0 mm; (*B*) ~ bregma − 2.0 mm. Locally elevated densities seen in, for example, the lateral septal nucleus (*LS*), the reticular nucleus of the thalamus (*RT*), and the amygdala (*AMY*). HG expression appears to be less concentrated and obtains lower levels overall in the ASD animal than in the control, but this remains a hypothesis to be subjected to quantitative testing on more animals.

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

P. Osten H. Ederle G. Fitzgerald Y. Kim C. Mende A. Narasimhan R. Palaniswamy E. Szelenyi N. Takada J. Taranda K.U. Venkataraju

We are interested in studying how brain circuits assemble during development and give rise to behavior in the juvenile and adult mouse, both under normal conditions and in genetic mouse models of autism.

Mapping Mouse Brain Activation

For the past several years, we have been developing a largely automated and highly quantitative approach to mapping neuronal activation in the mouse brain at cellular resolution. We have introduced a novel microscopy, called serial two-photon (STP) tomography, which achieves high-throughput fluorescence imaging of whole mouse brains by integrating two-photon microscopy and tissue sectioning (Ragan et al., *Nat Methods 9:* 255 [2012]; Osten and Margrie 2013). More recently, we have established a pipeline of computational methods that allows the visualization of the immediate-early gene *c-fos*, a molecular marker of neuronal activation, by STP tomography in transgenic c-*fos*-GFP mice. The c-*fos*-GFP-positive neurons are computationally detected, their distribution is warped to a reference brain registered to the Allen Mouse Brain Atlas (ABA), and the activated brain regions are identified by rigorous statistical tests comparing c-*fos*-GFP cell counts in more than 600 anatomical ABA regions (Fig. 1). We have

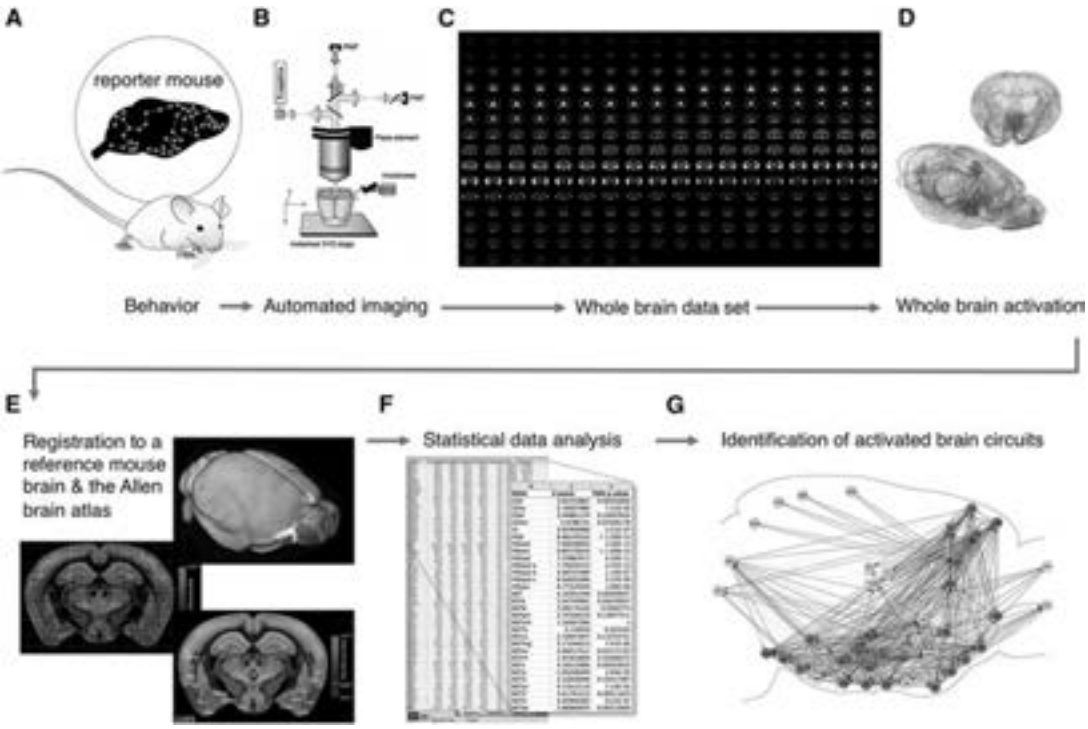

Figure 1. Mapping mouse brain activation by STP tomography. (*A*) Brain activation causes the induction of c-*fos*-GFP. (*B*–*C*) The brain is imaged by STP tomography. (*D*) c-*fos*-GFP cells are computationally detected. (*E*) The data sets are warped to a "reference" brain and Allen Mouse Brain Atlas. (*F*) The distribution of c-Fos-GFP cells is compared by statistical analyses, and the results are tabulated in a spreadsheet of brain regions. (*G*) Activated brain regions are plotted in circuits based on their known anatomical connectivity.

demonstrated the power of these methods by generating whole-brain activation maps for two complex behaviors: a brief social interaction between a male and a female mouse and an odor-driven novelty exploration (manuscript submitted for publication; see Fig. 2). We anticipate that our methods will enable routine quantitative generation of whole-brain activation maps representing different complex behaviors, including other innate behaviors (e.g., sex, aggression, and fear) and higher cognitive behaviors (e.g., decision-making). We predict that such maps will become a valuable resource for the systems neuroscience community. Furthermore, the application of our methods to the screening of brain activation in genetic mouse models of autism may reveal brain circuit deficits common to multiple susceptibility genes, which could serve as clinically relevant brain-circuit-based targets (or biomarkers) for the development of novel therapeutics.

Toward Quantitative Cell-Type-Based Mapping of the Mouse Brain

The mouse brain comprises ~70 million neurons and ~30 million glia and other cells. In addition to the method for mapping brain activation described above, we have also developed an assay for quantitative mapping of the many neuronal and glia cell types present in the mouse brain (see example in Fig. 3). This work aims to provide a complete picture of cell type distribution in the mouse brain during development, in the adult, and during aging. The study of the developing brain will shed light on the temporal sequence of cell-type- and region-specific circuit assemblies that give rise to emerging motor, sensory-perceptual, and cognitive specializations in the young brain. The experiments in the adult brain will focus primarily on the questions of gender differences in cell numbers that have been described for several

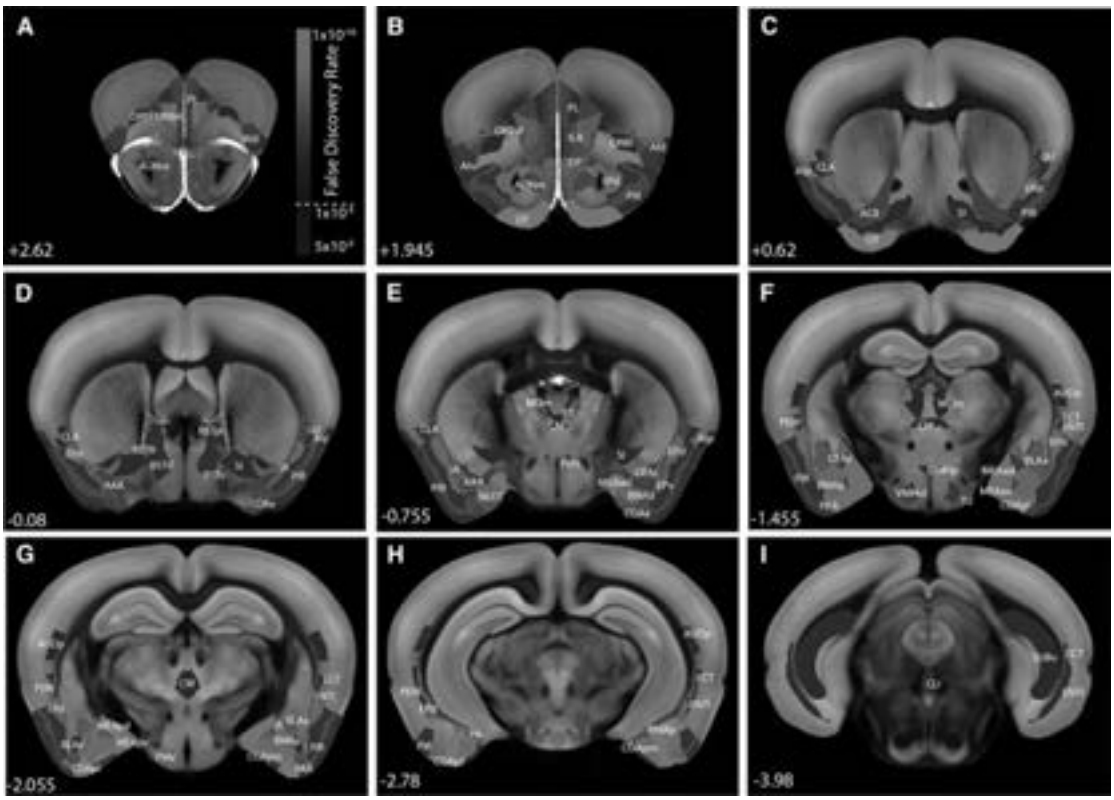

Figure 2. Mapping social behavior in the mouse brain. Brain areas activated selectively during social behavior are visualized in color according to statistical significance (*right* side in panel *A*) overlay on the reference brain volume (*gray*). The social brain circuit includes areas of the prefrontal cortex (*A–B*), regions of the ventral striatum and dopaminergic areas linked to reward processing (*C,I*), and areas linked to emotional processing, such as amygdala (*E–H*) and bed nuclei of the stria terminalis (*D*).

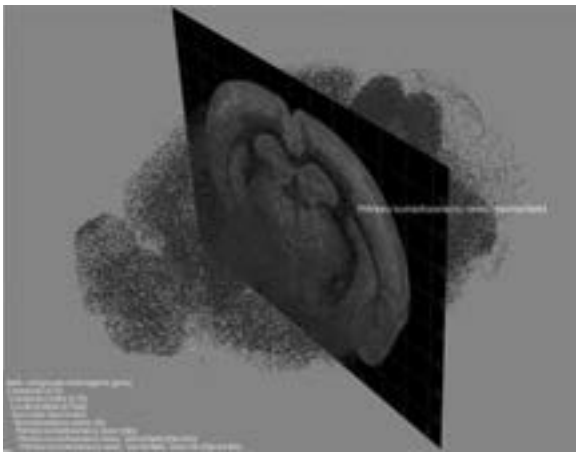

Figure 3. Whole-brain SOM+ interneuron distribution. Shown is an example of cell type distribution, specifically of the somatostatin-positive (SOM+) interneurons, in the adult mouse brain. The view shows a registration to the Allen Mouse Brain Atlas visualized in a single coronal plane.

brain areas but have not been examined at a whole-brain level. And, finally, the studies in older animals will aim to determine whether a loss of specific cell types may contribute to the decline in certain brain functions during aging.

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NEURAL CIRCUITRY FOR SOCIAL COMMUNICATION

S. Shea B. Cazakoff B. Lau
D. Eckmeier B. Schuman
G. Ewall K. Tranchina

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. We are particularly interested in how all stages of this decision arc are flexible according to experience and behavioral state.

The scientific benefit of this approach is twofold. First, we want to identify fundamental principles for how the circuitry of the brain adaptively controls complex behavior. In our pursuit of this goal, innate social behaviors are advantageous because they allow us to study neural circuits in light of problems they are exquisitely adapted to solve. Second, it is also our goal to pinpoint disturbances in the circuits that impair appropriate use of social information. Difficulty with social perception and cognition are core features of the autism spectrum disorders (ASD); for example, patients may have trouble 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 two broad areas of research in the lab. The primary arm of the lab's efforts is focused on neural circuits that facilitate detection, discrimination, and memories for olfactory cues. In particular, we are interested in how these circuits help an animal flexibly select appropriate behavioral decisions toward potential mates, rivals, offspring, predators, and food sources. Our approaches range from detailed in vivo synaptic physiology in anesthetized animals, to functional imaging and physiology in head-fixed mice, to recording and manipulating neural activity in awake, freely behaving animals. We ultimately hope to uncover the neural encoding of social cues during a live encounter with another mouse. The secondary arm of our research program is aimed at understanding the plasticity of cortical circuits that enable vocal communication between mice. Specifically, we have been working on how this circuitry and the attendant behavior are altered in mice that have impaired function of the gene *MeCP2*, which is mutated in humans with Rett syndrome.

State-Dependent Neuronal Coding in Granule Cells of the Olfactory Bulb

B. Cazakoff, B. Lau, K. Crump, H. Demmer

Arguably, the most critical and finely tuned instrument providing a mouse with information about its social world is its sense of smell. Detection and interpretation of olfactory stimuli in the brain begin at the olfactory bulb. Odor signals passing through the olfactory bulb are subject to processing and modification by the intrinsic neuronal circuitry of the bulb. Among these intrinsic neurons, inhibitory granule cells are certainly the most numerous, arguably the

most important, and yet somehow the most mysterious. These cells are the primary conduit for the olfactory bulb to integrate odor information with signals originating deep in the brain. Furthermore, granule cell anatomy along with a handful of functional studies suggests that they are crucial for olfactory discrimination and learning. Nonetheless, their activity patterns in vivo are poorly understood, and their electrophysiological properties in awake animals are completely unknown. This is because granule cells do not yield to conventional recording techniques. Former lab postdoc Dr. Heike Demmer therefore developed reliable methods for recording and labeling olfactory bulb granule cells. In a tour de force series of experiments, WSBS student Brittany Cazakoff, postdoctoral fellow Dr. Billy Lau, and CSHL URP Kerensa Crump applied these methods to mice that were awake with their heads fixed, but running freely on a foam ball and receiving water rewards from a lick tube. Their recordings represent the first reported view of the activity of this important cell type during wakeful behavior. The team's results conclusively show that granule cells become much more active during wakefulness and surprisingly uncouple from the animal's breathing and sniffing pattern. This seems to suggest that upon waking, granule cell activity becomes dominated by internal signals from the brain rather than the sensory properties of odors. If so, we have functionally identified a pathway central to the remarkably labile processing of odor stimuli. Ms. Cazakoff's thesis will be focused on expanding this paradigm to include discrimination of odors and associative learning to study how the granule cells' firing patterns reflect experience with and behavioral significance of an odor.

Noradrenaline Stores Olfactory Memories through Dynamic Regulation of Inhibition

H. Demmer, D. Eckmeier, B. Cazakoff [in collaboration with A. Koulakov, Cold Spring Harbor Laboratory]

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 of the responses to odorants in the olfactory bulb,

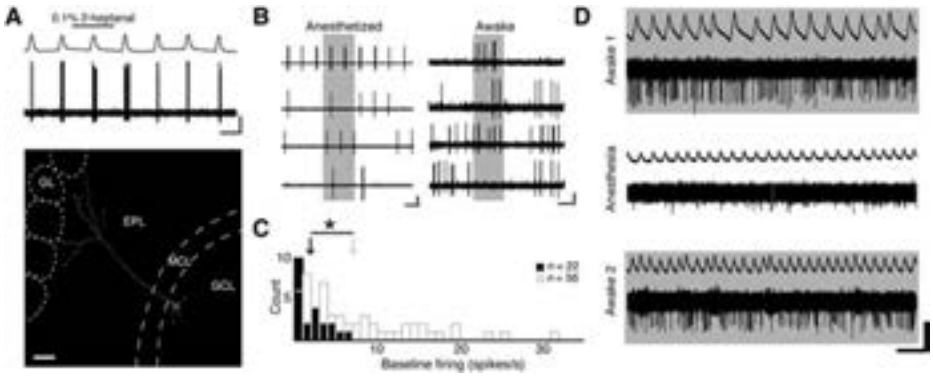

Figure 1. (*A*) Recording from identified MOB granule cells in the awake and anesthetized mouse. Representative recording from a GC in an anesthetized mouse showing a neuronal spiking trace and respiratory signal above. Scale bar, 2.5 mV/1 sec. (*Lower panel*) Photomicrograph of the corresponding neurobiotin-filled cell exhibiting hallmark GC features. Scale bar, 40 µm. (GCL) Granule cell layer; (MCL) mitral cell layer; (EPL) external plexiform layer; (GL) glomerular layer. (*B*) Representative traces showing the heterogeneity of odor responses in the awake state. In contrast to anesthetized state GCs, cells in the awake animals variably responded with activation (awake trace 1), delayed onset activation (awake trace 2), and inhibition (awake trace 4) in response to different odors. Scale bar anesthetized, 5 mV/1 sec. Scale bar awake, 2 mV/1 sec. (*C*) Distribution of spontaneous firing rates during anesthetized (black bars, mean ± S.D. = 1.90 ± 1.9 spikes/sec) and awake states (gray bars, mean ± S.D. = 7.41 ± 7.1 spikes/sec). Spontaneous activity of GCs is significantly increased in awake animals (Mann–Whitney U test, $p = 0.00012$). (*D*) In select cases, individual cells were recorded during both anesthesia and wakefulness. Spontaneous firing decreases when isoflurane is administered (*unshaded trace*) and returns to preanesthesia level when anesthesia is turned off. Scale bar, 1 mV/1 sec.

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 have been exploiting the intimate relationship among NA, olfactory bulb activity, and behavior to trigger and observe olfactory memories in mice. We discovered that indeed, when NA release is evoked by stimulating locus coeruleus, the source of most NA, while the sleeping mouse sniffed an odorant, neural responses to that odor underwent specific long-term alterations. Remarkably, once awake, the mouse's subsequent behavior toward the odorant was also changed. In other words, the mouse seemed to remember the odor and treat it as though it were familiar. This finding has motivated several of our studies into how NA causes lasting changes to odor processing circuitry.

Memories are widely believed to be stored as changes to the synaptic connections among the neurons in our brains. How are olfactory memories for individuals stored mechanistically among the specific synaptic connections of the various neuronal types in the olfactory bulb? In one set of experiments aimed at answering this question, Dr. Heike Demmer used her techniques for recording granule cells to observe their activity during the induction of NA-dependent plasticity. She found that naturalistic stimulation of NA release such as occurs in social encounters suppresses granule cells. Suppression of granule cells transiently increases the excitability of mitral cells by relieving them from inhibition. This transient release of mitral cells from granule cell control is a key trigger for the synaptic plasticity that likely underlies memory. Indeed, this disinhibitory event triggers selective long-term changes to mitral cells that signal the presence of the learned odor. We have therefore pinpointed the synaptic connections between granule cells and mitral cells as an important synaptic substrate of individual recognition memories.

Dr. Dennis Eckmeier has been taking a different approach to observe population mechanisms of NA-dependent memory formation with functional neural imaging. He uses a technique in which the sensory neurons that provide input to the olfactory bulb are labeled with a fluorescent activity sensor that allows him to monitor the strength of activation in foci called "glomeruli." By comparing the response strength of each of these glomeruli before and after NA release, Dr. Eckmeier has shown that NA weakens the response to paired odors. This effect is only observed in cases where NA is released during the odor by stimulation of a brainstem structure called locus coeruleus (LC). These data establish the synaptic input to the MOB as a target of noradrenergic modulation, thus revealing that the effects of learning and plasticity in the olfactory system extend to remarkably early sensory signaling events.

Vocal Communication Is Impaired in a Mouse Model of Rett Syndrome

B. Lau, G. Ewall [in collaboration with J. Huang and K. Krishnan, Cold Spring Harbor Laboratory]

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 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. Moreover, females who have never given birth do not innately show approach responses to pups or their calls; however, they can learn to perform the behavior with experience.

Dr. Billy Lau is collaborating with CSHL professor Dr. Josh Huang and his postdoctoral fellow Dr. Keerthi Krishnan to examine how vocal perception of pup calls is affected in mice that are missing one copy of a gene called *MeCP2*. Impairments in the function of this gene are understood to cause the autism spectrum disorder Rett syndrome, and the Huang lab's findings regarding mutations in *MeCP2* suggest that they may affect inhibitory networks in the auditory cortex important for development of pup call sensitivity. Indeed, we find that females which possess only a single copy of *MeCP2* are not able to develop proficiency at gathering pups. This is an important finding because we describe the first robust

behavioral phenotype to be observed in mice that most closely genetically resembles humans with Rett syndrome. It is also significant that this phenotype is a social communication deficit. Ongoing studies are exploring the molecular and neurophysiological effects of *MeCP2* mutations on circuits in the auditory cortex that facilitate detection of pup calls. Furthermore, we are using this novel behavioral assay to assess strategies for ameliorating the effects of Rett syndrome.

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 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.

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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? 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: 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 in humans.

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? Ultimately, what are the roles of the many genes implicated in learning on network-level activity in the brain?

A Population Code for Odor Identity in the *Drosophila* Mushroom Body

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 features 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 the following contexts: (1) when flies learn fine discriminations between highly similar odors and (2) when they learn an association with one odor, and then 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 odor. We compared this behavioral measure with the specificity of the response pattern of ~150 MB neurons to these odors. We found that we could use the MB activity patterns to accurately predict the specificity of memory formation subsequently measured in behavioral experiments.

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. We have also used the activity patterns we measured in the MB to predict generalization of odor memories.

There are two important conclusions from this study: (1) Analog differences between odor representations in the olfactory receptor neurons are converted to binary differences in 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.

Dendritic Claws of Mushroom Body Neurons Integrate the Olfactory Code

E. Gruntman

How is it possible to recognize an odor as a unified smell (e.g., coffee) when it has a large number of different components, which in turn have many different chemical features? One idea is that olfactory receptor neurons (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. But are there neurons at deeper layers of the brain that read this combinatorial code and respond to specific combinations of co-active inputs? In this work, we show that the integration of these different inputs in the fly occurs on the dendritic trees of individual MB neurons.

We addressed this issue by examining odor responses of individual dendritic input sites, making this one of 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 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.

To evaluate how these inputs are integrated, we used optogenetic techniques to control the activity of the input neurons. Our results provide an elegant explanation for why MB neurons are so odor-selective. Not only do they receive different types of inputs, but they require those different inputs to be co-active in order for the MB neuron to spike. Examining the time course of synaptic summation showed a rapid initial depolarization, which quickly plateaued at a level well below spike threshold. Summation of inputs from different dendritic sites is also strongly sublinear. Nevertheless, if sufficient numbers of different input sites are activated, membrane potential can reach spike threshold. Contrary to prevailing models for generating stimulus-specific responses, which rely mainly on supralinear summation of coincident inputs, our results show a surprising variant: a demand for strong multiple concurrent inputs due to sublinear summation.

Dopamine Controls the Signal-to-Noise Ratio of Transmission through the Mushroom Body

T. Hige, K. Honegger

Neuromodulators such as dopamine and octopamine are essential for learning, but how they act and what their precise role is during the learning process has not been established. We examined the effects of these neuromodulators on activity in the MB. Surprisingly, we found that dopamine affects the signal-to-noise ratio of odor representations in the MB. Preliminary results suggest that dopamine increases the reliability of MB responses. Reliability is difficult to achieve in sparse representations, because neurons typically respond with small numbers of spikes. Thus, it seems particularly useful for the circuit to use neuromodulators to increase signal to noise depending on the behavioral context the animal is in; essentially, dopamine could control the salience of a stimulus.

In addition, we have shown that dopamine diminishes odor responses in the neurons that are downstream from the MB. Together, these results suggest a model where the dopamine acts to make the circuit a selective filter, increasing the signal-to-noise ratio of responses in the MB while decreasing signal transmission through the MB. These results may be an excellent entry point for studying the fundamental cognitive process of attention, which is essentially a process of filtering out distracting information to focus on pertinent inputs. We are now testing this possibility and investigating the precise mechanisms of the dopamine effects.

Mushroom Body Output Neurons and Odor Categorization

T. Hige

Almost no studies have examined what happens to sensory information downstream from areas with sparse representations. So what happens to odor information after

it leaves the MB? In collaboration with Gerry Rubin's lab at Janelia Farm, we have functionally characterized odor coding in the complete set of 34 MB output neurons (MBONs). The following are our main findings:

1. ***Synaptic plasticity diversifies MBON tuning properties.*** We found that uniquely identifiable MBONs have strikingly different odor response properties across different flies. To test whether these individual differences are experience-dependent, we examined odor tuning of the same neuron across the two hemispheres of the brain. Identical MBONs in the two hemispheres have identical odor tuning properties; however, the tuning of this neuron is very different across different individual flies. This result suggested that MBON tuning in each fly is partly a product of that individual's developmental and experiential history. We tested this by showing that a central gene in learning and memory, *rutabaga,* is required for these individual differences to arise. This experiment is one of the first to show that the odor tuning properties of MBONs are shaped by synaptic plasticity.
2. ***MBONs represent categories/groups of odors.*** We examined odor responses across the entire population of MBONs and found that MBONs do a poor job of representing odor identity, especially in comparison to the upstream neurons that constitute the MB itself. This is because certain odors evoke extremely similar patterns of response across the MBON population. In fact, only three main groups of odors were identified based on the pattern of activity in the MBONs. Interestingly, one group was composed of odors that are repellent to flies, whereas another group contained food-based odors. This is an exciting result because few investigators have considered how different sensory inputs can be categorized into behaviorally meaningful classes. These results suggest that olfactory information is shaped into behaviorally relevant neuronal signals at this layer, an important step in the process of connecting sensory input to motor output.

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CIRCUITRY OF UNDERLYING CORTICAL PROCESSING AND DECISION-MAKING

A. Zador B. Burbach, W. Donovan, D. Gizatullina, S. Jaramillo, J. Kebschull, S.-G. Koh, U. Livneh, F. Marbach, H. Oyibo, I. Peikon, A. Reid, Q. Xiong, H. Zhan, P. Znamenskiy

My laboratory is interested in how neural circuits underlie cognition, with a focus on the cortical circuits underlying auditory processing and decision-making. To address these questions, we use a combination of molecular, electrophysiological, imaging, and behavioral approaches.

Characterizing Transposable Element Activity in TDP-43-Pathology Neurodegenerative Disorders

W. Donovan [in collaboration with J. Dubnau, Cold Spring Harbor Laboratory]

Mutations of the TAR DNA-binding protein 43 (TDP-43) have been linked to both amyotropic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD), debilitating neurodegenerative diseases for which there are no cures, effective treatments, or even diagnostic biomarkers. The mechanism by which TDP-43 proteinopathy causes neurotoxicity is not known. The main goal of this project is to test the hypothesis that retrotransposons are induced by TDP-43 pathology. To accomplish this, we are using the CRISPR (clustered regularly interspaced short palindromic repeats) genome editing system to generate a new mouse model in which the endogenous mouse gene contains disease-causing amino acid substitutions. We will validate the relevance of our hypothesis by examining TE expression in human postmortem tissue. Finally, we will use cell-culture-based assays to test whether TDP-43 has an impact on retrotransposon replication and whether this involves non-cell-autonomous effects that might explain the focality and spread of neurodegenerative disorders.

Circuits Underlying Auditory Representations and Decisions

S. Jaramillo, U. Livneh, F. Marbach

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. We use this approach to study the role of the auditory thalamus, cortex, and striatum in these behavioral processes. Using tasks in which the stimulus–action associations vary within a behavioral session, we have uncovered striking similarities in how thalamic and cortical neurons are modulated by the animals' choices.

Sequencing the Connectome

I. Peikon, D. Gizatullina, J. Kebschull, H. Oyibo

We are developing an entirely novel approach to mapping neural connectivity which harnesses the high-throughput power of next-generation sequencing. We have engineered a suite of molecular tools to barcode individual neuronal connections (BOINC) for read-out via DNA sequencing. By mapping neural connectivity onto DNA sequencing, we have rendered neural circuit reconstruction tractable with current techniques.

Interhemispheric Connections in the Auditory Cortex

S. Koh

The callosal projection is a long-range connection between the left and right auditory cortex, and it is one

of the major corticocortical projections in the auditory cortex. However, its role in auditory coding is unclear. We are studying how manipulating callosal projection affects auditory representations in the cortex. We found that silencing the auditory cortex in one hemisphere suppresses sound-evoked activity in the other hemisphere. The suppression effect was stronger for late response with latency longer than 40 msec and also for the response to sound coming from ipsilateral locations. Our results suggest that the callosal projection is well suited for slow interhemispheric integration of location-relevant information.

Role of Inhibitory Interneurons in Auditory Cortex Function

A. Reid

Fast synaptic inputs to neurons in the auditory cortex are either inhibitory or excitatory. Cortical interneurons are tremendously diverse. One inhibitory interneuron subclass, defined molecularly by the expression of parvalbumin ("PV+"), seems to be ideally positioned to mediate the fast component of the characteristic barrage of inhibition elicited by a sound. We are testing the hypothesis that PV+ inhibitory interneurons mediate fast sound-evoked inhibitory synaptic currents in the auditory cortex. Our proposal seeks to establish a causal link between a physiological property—the fast sound-evoked inhibition that contributes to receptive field dynamics—and a component of the underlying cortical circuitry. We approach the problem at three different levels, from brain slices through in vivo physiology to behavior. Although we are currently focusing on the role of one particular interneuron subclass (PV+), our approach 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.

Role of Corticostriatal Plasticity in Auditory Decisions

Q. Xiong, P. Znamenskiy

Corticostriatal plasticity has a key role in reinforcement learning, but how associations between stimuli and motor responses are established remains unclear. Recent work from our group demonstrated a causal role of corticostriatal neurons in driving choices during an auditory discrimination task, inspired by the classic random dot motion task used by Newsome and colleagues in macaques, in which subjects were required to choose the left or the right response port depending on the perceived frequency of a sound. We are testing the hypothesis that changes in the strength of corticostriatal synapses underlie the association between sound and action required to perform this task. Our results indicate that changes in the strength of a specific subset of corticostriatal synapses encode the arbitrary association between stimulus and motor response. Because all sensory cortical areas send projections to the striatum, our findings suggest a general mechanism for the formation of arbitrary sensorimotor transformations.

Labeling Specific Neuronal Subtypes by Ribozyme Mediated *Trans*-Splicing

H. Zhan, I. Peikon

We are developing a novel strategy to label specific neuronal subtypes by using ribozyme-mediated *trans*-splicing to couple recombinase (e.g., Cre) mRNAs into endogenous target genes (e.g., somatostatin or parvalbumin), mRNA and the expression of recombinase in specific neuronal cells being switched into the expression of an exogenous transgene such as green fluorescent protein (GFP) or red fluorescent protein (RFP). Our approach, if successful, has the potential to allow monitoring and manipulation of specific cell types even in model systems, such as rats and primates, that are not as genetically tractable as mice.

PUBLICATIONS

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong J. Beshel Y. Shuai
S. Khurana C. Xu

Food Odor Value Coding in the Fly Brain via Neuropeptide Y Homolog

J. Beshel

Chemosensation is a critical regulator of food-seeking behaviors across animal species. Food odor alone can trigger the appropriate approach for investigative behaviors essential for survival. Yet very little is known about how the brain determines odor value, let alone of potential food sources specifically. Our analysis of the *Drosophila* brain extends the role of a small number of hunger-sensing neurons to include food-odor value representation. In vivo two-photon calcium imaging shows that the amplitude of food odor-evoked activity in neurons expressing *Drosophila* neuropeptide F (dNPF), the neuropeptide Y homolog, strongly correlates with food-odor attractiveness. Hunger elevates neural and behavioral responses to food odors only, although heightened responses also exist for some food odors when fed. Inactivation of a subset of dNPF-expressing neurons or silencing dNPF receptors abolishes food-odor attractiveness, whereas genetically enhanced dNPF activity not only increases food-odor attractiveness, but also promotes attraction to aversive odors. Varying the amount of presented odor produces matching graded neural and behavioral curves that can function to predict preference between odors. We thus demonstrate a possible motivationally scaled neural "value signal" accessible from uniquely identifiable cells.

The Mushroom Body Circuit Underlying Fruit Fly Forgetfulness

Y. Shuai, A. Hirokawa

Like memorization, forgetting is also a delicately organized process at both the molecular and neural circuit levels. We have been studying forgetting mechanisms using a relatively simple model organism, the fruit fly. Although flies readily learn to avoid odorants paired with a mild foot shock punishment, the learned aversion declines substantially in a few hours. We previously showed that this rapid early memory forgetting is tuned by a cytoskeleton-regulating molecular cascade headed by small G protein Rac. Rac function was mapped to the mushroom body (MB) γ lobe neurons. This subset of MB intrinsic neurons comprises ~700 cells in each hemisphere and sends parallel axons to the MB medial lobes, where they are intersected by a few dozen of the so-called extrinsic (MBex) neurons from heterogeneous sources. How these MBex neurons are involved in forgetting is not clear. We thus screened ~40 MBex Gal4 drivers, which together shall cover most types of the MBex neurons connected to the medial lobes. A few of them, when coupled with neuron-silencing genetic tools, showed memory enhancement, indicating that they are part of the MB forgetting circuit. In particular, three of these Gal4s label a common single pair of neurons, which we named the MB-M9 neurons. MB-M9 appear to be feedback neurons that receive input from one specific axonal compartment of the γ lobe, γ4, and project back to other axonal compartments of the γ lobe and to areas outside the MB as well. We are in the process of detailing the anatomy of MB-M9 and evaluating their interactions with other MB neurons. We hope that these efforts will shed light on the organization of the MB circuit underlying fruit fly forgetting.

NF1 and Memory in *Drosophila*

C. Xu

Neurofibromatosis type 1 (NF1) is a genetic disorder affecting one in 3500 people in the population. Affected individuals manifest overgrowth of various types of tissue and some patients show cognitive defects. The mutated gene responsible for NF1 disorder is called neurofibromin and it is conserved among

species. When the neurofibromin gene is deleted, the *Drosophila* animal model has both learning and long-term memory defects in olfactory aversive conditioning. In contrast to the aversive conditioning, the appetitive conditioning is more effective in forming long-term memory by single training trails, whereas aversive conditioning needs multiple training trails. Here, we showed that neurofibromin is also required for normal appetitive memory performance. We are currently mapping the circuits in which neurofibromin is involved to maintain normal appetitive memory.

Decision-Making in *Drosophila* Larvae

S. Khurana

In the previous year, we initiated the development of an image acquisition, tracking, and analysis system to measure the behavioral responses of animals. We have specifically focused on the larvae of *Drosophila melanogaster* because of the genetic toolkit of the fruit fly. The two-dimensional locomotion of larvae, compared with the three-dimensional movement of an adult fly, makes the tracking relatively straightforward. Additionally, larvae have a much smaller number of neurons than the adult fly, making the problem of neural mapping of behavior potentially more tractable. This year, we completed the development of the first iteration of the tool and are working to make it an online-accessible package that can be easily modified for many other tracking and analysis purposes. The advances of our analysis compared to other contemporary such methods was our ability to look at the sequence of patterns in behavior, instead of simply looking at the isolated features. Additionally, we can track multiple animals at the same time, increasing the efficiency of measures. Using this method, we have looked at the conditioned behavior of larvae to ask if there is a difference in behavioral sequences between innate and acquired responses to odors. Our results show a severalfold increase in the frequency and duration in the slow phases (when animals slow down and frequently change the direction of their locomotion) when animals make a choice to approach or avoid the conditioned odor over such decisions in innate response. Whether this is entirely capturing the sequence of the decision-making behavioral phenotype in larvae or whether our measurements contain some noise arising from stimulus concentration issues currently remains to be settled. The long-term goals of this line of work are to create a more efficient drug and more mutant screens and to improve behavioral measurements in general.

PUBLICATIONS

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PLANT BIOLOGY

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 gene encoding a chaperonin, *CCT8*, that controls the transport of a transcription factor SHOOTMERISTEMLESS (STM) between cells in the plant stem cell niche, or meristem. STM is critical for stem cell maintenance, and studies of the *CCT8* gene indicate that movement of STM 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, and their work has implications for crop yields. Recent examples include discovery of a subunit of a heterotrimeric G protein that is conserved throughout animals and plants, and their studies indicate that this gene controls stem cell proliferation. They have found that in plants, the G protein interacts with a completely different class of receptors than in animals. Their discovery helps to explain how signaling from diverse receptors is achieved in plants. This year, they also demonstrated that weak mutations in one of the receptor proteins can enhance seed production in maize, which could lead to yield increases. Separately, the lab has characterized system-wide networks of gene expression, using "next-gen" profiling and chromatin immunoprecipitation methods that have revealed many new hypotheses in developmental networks controlling inflorescence development. They are also developing a collection of maize lines that can drive expression of any reporter or experimental gene in any tissue type—tools of great interest to maize researchers that are being made available to the broader scientific community, enabling experiments never before possible in crop plants.

Zachary Lippman's research focuses on the process of flowering and flower production, which is a major contributor to plant reproductive success and agricultural yield. By identifying genes that control how tomato plants produce their flowers in their characteristic repeated zigzag arrangement (e.g., tomatoes on a vine), Lippman's lab is addressing when and how flowering branches known as inflorescences develop on plants, particularly fruit-bearing plants. Of particular interest is how these "reproductive phase transitions" have contributed to the evolution of diverse inflorescence branching patterns in tomato's larger Solanaceae family, which includes plants that make just one flower, such as pepper and petunia, in each inflorescence, to plants whose inflorescences produce dozens of branches and hundreds of flowers, such as many wild species of tomato. Using a combination of genetic, genomic, and molecular approaches, 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. Already, his work on genes that are responsible for the production and activity of a universal flowering hormone known as florigen has resulted in novel approaches to fine-tune plant architecture and flower production, boosting yield beyond leading commercial varieties. To continue hunting for new genes, Lippman has adopted a systems-biology approach and next-generation sequencing technology to capture those genes that are active as stem cells mature from a vegetative to a reproductive state. Nearly 4000 genes were found to reflect the existence of a "maturation clock," and one of the clock genes known as *Terminating Flower* acts as a key regulator to maintain a progressive pace to flowering—which in turn, dictates how many flowers are produced on each tomato inflorescence. Finally, the Lippman lab determined the genome sequence of the "currant tomato," the wild ancestor of larger-fruited cultivated tomatoes, in order to better understand how flower and fruit production changed during the process of crop domestication.

Epigenetic mechanisms of gene regulation—chemical and conformational changes to DNA and the chromatin that bundles it—have had an important impact on genome organization and inheritance and on cell fate. These mechanisms are conserved in eukaryotes and provide an additional layer of information superimposed on the genetic code. **Robert Martienssen**, a pioneer in the study of epigenetics, investigates mechanisms involved in gene regulation and stem cell fate in yeast and model plants including *Arabidopsis* and maize. He and his colleagues have shed light on a phenomenon called position-effect variegation, caused by inactivation of a gene positioned near densely packed chromosomal material called heterochromatin. They have discovered that small RNA molecules arising from repeating genetic sequences program that heterochromatin. Martienssen and colleagues have 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. They found that some of these instructions, or epigenetic marks, could be inherited in the next generation. 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. The lab has also shown that when RNA polymerase II has transcribed a stretch of DNA, the RNA interference 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. This year, as part of a collaborative project to generate a high-quality full genome map of the oil palm plant, Martienssen and his colleagues identified a single gene that controls the yield of oil palm trees. This discovery will increase yields and should lessen the environmental burden of oil palm production, which often threatens already endangered rainforest lands.

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, such as leaves, arise. **Marja Timmermans** and colleagues are studying the genetic networks that regulate plant stem cell activity. Using genomic approaches, they have defined gene expression signatures that distinguish indeterminate stem cells from their differentiating derivatives. They have also worked out the mechanism that suppresses stem cell fate to allow cells to differentiate and have shown that this process requires a highly conserved epigenetic gene silencing mechanism. In particular, Timmermans' group has shown that specific DNA-binding proteins mediate the recruitment of Polycomb repressive complexes to stem cell factors, an action that stably represses their expression in differentiating organs. This work addresses a major unresolved question in the field of epigenetics: how Polycomb proteins, which do not bind DNA themselves, recognize defined targets. Plant stem cells also produce signals important for the patterning of lateral organs. The lab has discovered that small RNAs can traffic from cell to cell and are among the stem-cell-derived signals. They have found that polarity in leaves is established via opposing gradients of mobile small RNAs that act as morphogen-like signals. Their most recent findings identified a third small RNA gradient involved in maintenance of organ polarity. These findings illustrate the complexity with which small RNAs generate developmental patterns. Currently, they are investigating parameters of small RNA mobility and the unique patterning properties of resulting small RNA gradients. Mathematical modeling predicts that such gradients might serve to generate robustness during development.

DEVELOPMENTAL BIOLOGY: STEM CELLS, SIGNALING, AND CONTROL OF PLANT ARCHITECTURE

D. Jackson R. Balkunde, A. Eveland, S. Hiraga, B. Il Je, T. La Rue, S. Lee, I. Liao, A. Masson, A. McGarry, M. Pautler, E. Shea, H. Thanh Bui, K. Vera, S. Vi, R. Willen, Y.G. Wolfenson, Q. Wu, 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 that passes between cells and tissues. We are particularly interested in discovering the signals that carry this information, in finding out how the signals are transmitted, and how they function. In one project, we 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. We are using genetic screens to identify novel factors that control the transport of a transcription factor, SHOOTMERISTEMLESS (STM), between cells in the plant stem cell niche, or meristem. STM is critical for stem cell maintenance, and our studies indicate that movement of STM between cells is required for this function. We also continue 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 our studies indicate that this gene controls stem cell proliferation. In animal systems, the G protein interacts with a class of receptors that are called GPCRs (G-protein-coupled receptors), but we have found that in plants, the same protein interacts with a completely different class of receptors. Our discovery helps explain how signaling from diverse receptors is achieved in plants. This past year, we also demonstrated that weak mutations in one of the receptor proteins can enhance seed production in maize, which could lead to yield increases. We have also identified new genes that control maize ear development and are busy figuring out their mode of action. Separately, our lab has characterized system-wide networks of gene expression in inflorescence development, using "next-gen" profiling methods, and we are 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.

Regulation of Cell-To-Cell Trafficking of the KNOTTED1 Transcription Factor

R. Balkunde, H. Bui

Cell-to-cell communication functions in specifying cell fate and coordinating development in all multicellular organisms. However, unlike animal cells, plant cells have cell walls that could limit exchange of signal molecules between neighboring cells. Plants have therefore developed symplasmic connections by means of special structures called plasmodesmata (PDs). PDs allow the selective trafficking of signaling macromolecules, such as transcription factors and RNAs. In addition to cell-fate specification, PDs are 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 PDs more than 100 years ago, the underlying components and mechanisms of PD trafficking remain poorly understood. Hence, we are taking an unbiased genetic strategy to dissect these molecular mechanisms, using a reporter system involving *Arabidopsis* leaf trichomes as an easily scorable phenotype and the developmentally important mobile proteins KNOTTED1 and its *Arabidopsis* homolog SHOOTMERISTEMLESS.

The trichome rescue system designed by our lab is widely used to characterize the mobility of a protein. Briefly, GL1 is required for trichome formation in the *Arabidopsis* leaf epidermis, and it is cell autonomous. Therefore, GL1, when expressed in mesophyll in *gl1* mutants, does not rescue trichomes. However, when fused to KN1 (GL1-KN1), it now results in trichome rescue, as the mobile KN1 brings GL1 into the epidermis. Ethylmethanesulfonate (EMS) mutagenesis screening using this system, followed by Illumina high-throughput sequencing, has previously resulted in identification of a mutation in a gene encoding CCT8, a chaperonin subunit. This led to an exciting discovery that chaperonin facilitates KN1 cell-to-cell trafficking and stem cell maintenance in *Arabidopsis*, which clearly supports the functional relevance of chaperonin-mediated trafficking through PD. These results highlight the importance of protein conformational changes for PD trafficking.

Current efforts are focused on understanding the mechanism of CCT8 action and identifying new trafficking regulators. Our efforts have resulted in the identification of potential mutants with interesting developmental defects, in addition to loss of trichome rescue. For example, mutant 1058 has leaf-shape defects, and mutant 1066 has narrow and elongated leaves and an enlarged vegetative meristem (Fig. 1). We have mapped these mutants to a region of ~0.7 Mb on the upper arm of chromosome 3 (1058) and lower arm of chromosome 4 (1066) using classical marker-based mapping. We next identified potential candidates within these mapping regions using next-generation sequencing. We found four novel single-nucleotide polymorphisms (SNPs) in coding regions within the mapping region for each mutant. Interestingly, some of the putative candidates have been reported in the PD proteome. The putative candidates also include genes in families that have been implicated in trafficking through PD. Currently, we are confirming the causal mutations using T-DNA insertional mutants and by rescue experiments using transformation compatible bacterial artificial chromosomes (BACs) (TAC) clones. Once we have identified the candidates, we will proceed with the detailed characterization of the mechanisms and their biological significance in plant growth and development in the context of developmental defects observed in our mutants.

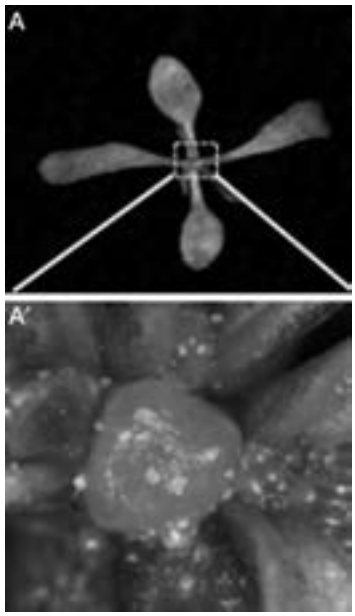

Figure 1. Trafficking mutant 1066 has developmental phenotypes and loss of trichomes (*A*). (*A′*) Enlarged image of inset marked in *A*, showing enlarged meristem.

Role of PDLPs in Cell-To-Cell Communication and Meristem Development

H. Bui, K. Vera

PDs are channels connecting adjacent plant cells. These channels are formed during cell division, when strands of endoplasmic reticulum are trapped in the cell wall formed between daughter cells. Many development regulatory proteins, including transcription factors (TFs), are found to act non-cell autonomously by moving through PDs. Classic examples included the homeobox TF KN1, the GRAS TF SHORTROOT (SHR), the myeloblastosis (MYB) TF CAPRICE (CPC), the WD40 protein TRANSPARENT TESTA GLABRA1 (TTG1), and FLOWERING LOCUS T (FT). Although KN1 and SHR are required for proper development and morphogenesis in the shoot and root. respectively, TTG1 is important for trichome and root hair patterning, and FT regulates flowering induction. Intercellular trafficking of these proteins is required for their function.

Despite the importance of targeted PD trafficking in plant development, little is known about the

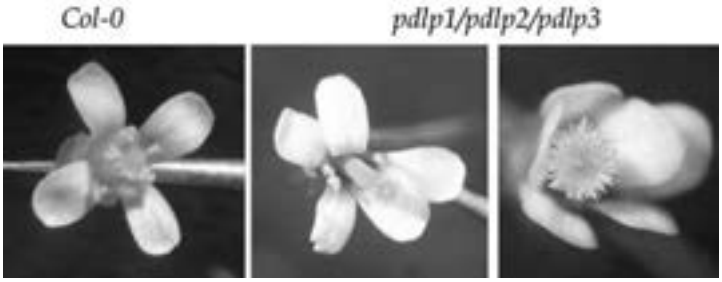

Figure 2. Abnormal floral developmental phenotype in *pdlp* mutants. *Col-0,* wild type. *pdlp* triple mutants have additional floral organs, suggesting a meristem defect.

mechanism of this process. PD resident proteins and PD trafficking regulators have been identified in genetic and proteomic screens. Our research focuses on studying a family of eight PD-localized proteins (PDLP 1–8) identified in a proteomic study of the PD-enriched membrane fraction. Interestingly, *PDLP2* and *PDLP3* are expressed in specific domains in the shoot meristem, raising a question about their roles in trafficking of stem-cell regulators, especially KN1 and STM. Moreover, we found that the triple *pdlp* mutant (*pdlp1/pdlp2/pdlp3*) exhibits interesting petal number phenotype (Fig. 2), suggesting that PDLPs are also important for floral development. We aim to elucidate the role of PDLPs in regulating protein trafficking in the plant stem cell population. This study, along with genetic screen using the trichome rescue system, will provide insight in to the general mechanism of intercellular trafficking and, more specifically, in to its role in stem cell development, which will have broad significance and relevance to all multicellular systems.

Generation of a pOp-LhG4 *trans*-Activation System for High-Throughput Protein Localization and Functional Studies in Maize

Q. Wu, T. Zadrozny [in collaboration with A. Chan, J. Craig Venter Institute; A. Sylvester, University of Wyoming]

Recent advances in sequencing technology have generated genome sequences from nearly all groups of organisms. These massive data sets have revolutionized the types of biological questions that can now be addressed. Despite the ability to predict the function of some proteins by comparative bioinformatics, experimental validation remains necessary to assign function definitively. One effective approach to validate gene function is to express the gene tissue or cell specifically. Currently, we are developing a pOp-LhG4 *trans*-activation system in maize that allows researchers to express genes of interest in different tissue or cell types.

The pOp-LhG4 system includes (1) a chimeric promoter, pOp, that consists of *lac* operators cloned upstream of a minimal CaMV promoter and (2) a transcription activator, LhG4, which is a fusion between a high-affinity DNA-binding mutant of lac repressor, *LacI*^{His17}, and transcription activation domain II of GAL4 from *Saccharomyces cerevisiae.* The pOp promoter is not activated in the reporter lines until crossed with activator lines that express LhG4 driven by appropriate tissue-specific promoters. Thus, the pOp-LhG4 *trans*-activation system allows specific expression or missexpression of any gene of interest. Using our experience in maize promoters, we are now developing different driver and reporter lines in maize that enable precise experimental intervention to study gene function. We have already generated several constitutive, meristem, embryo, root, and leaf-specific LhG4 drivers, including pEF1A, p*UBIQUITIN*, p*RAMOSA3*, p*WUSCHEL, pCRWAQ81, pSUT1, pSH1, pGBL1,* and p*YABBY14* (Fig. 3), as well as a pOp responder line, pOp-ZCN8::YFP (maize homolog of flowering inducer, FT), as a means to test the specificity of the system with a readout in flowering time. We found that neither the driver plants, pEF1A::LhG4, nor the responder plants, pOp::ZCN8-YFP, showed an early-flowering phenotype, whereas the F1 plants containing both pEF1A::LhG4 and pOp::ZCN8-YFP showed early flowering (Fig. 3). We are now working to create additional tissue-specific LhG4 driver lines and responder lines. Our goal is to produce an array of cell- and tissue-specific LhG4 lines,

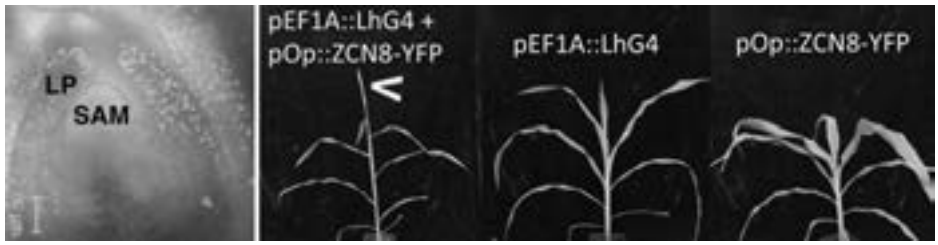

Figure 3. Maize transactivation system. (*Left-most panel*) pYabby14::LhG4//pOp::TagRFP-T specifically expresses in leaf primordia (LP, white dots = nuclei) and not in the shoot apical meristem (SAM). (*Right panels*) A plant containing both pEF1A::LhG4 and pOp::ZCN8-YFP constructs showed an early-flowering phenotype (arrowhead points to tassel).

which we will use to (1) isolate specific cell types by fluorescence-activated cell sorting (FACS) for transcriptional profiling and (2) drive the missexpression of developmentally regulated genes 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.php

Control of Shoot Meristem Size and Phyllotaxy by the Maize Glutaredoxin *Abphyl2*

F. Yang, H. Thanh Bui, M. Pautler [in collaboration with V. Llaca and H. Sakai, DuPont Crop Genetics, Wilmington, Delaware]

Phyllotaxy is a major taxonomic indicator and it affects plant architecture and light capture efficiency. Maize *abphyl* (*abph)* mutants change phyllotaxy from alternate to decussate and develop an enlarged shoot apical meristem (SAM). We describe here a new dominant phyllotaxy mutant, *Abph2*. *Abph2* mutants have an enlarged SAM and a decussate leaf pattern that becomes visible at leaf ~4–5 stage. Map-based cloning brought *Abph2* into a region of ~20 kb on chromosome 7, containing five predicted genes in the reference B73 genome. However, direct sequencing, as well as transcript analysis by reverse transcriptase–polymerase chain reaction (RT-PCR), did not give any obvious clues as to the identity of the gene. Therefore, a BAC library generated from the *Abph2* mutant was screened using probes located within the 20-kb mapping interval. BAC sequencing revealed a 4.5-kb fragment inserted into the mapping interval. This inserted fragment contained a predicted glutaredoxin gene identical to a gene (named *MSCA1*) located ~800 kb upstream. A transgenic line containing this 4.5-kb fragment fused with yellow fluorescent protein (YFP) tag phenocopied the dominant *Abph2* phyllotaxy defect, showing that the inserted glutaredoxin gene (named *Abph2* hereafter) is the cause of the decussate leaf phenotype. Meanwhile, putative knockout lines of the dominant *Abph2* mutation were screened by EMS mutagenesis. Sequencing the *Abph2* gene in these knockout lines found point mutations leading to conserved amino acid changes in each line, further pinpointing the inserted glutaredoxin gene as *Abph2*. RNA in situ hybridization indicated that the *Abph2* transcripts accumulate in P_0 leaf primordia and vasculature. However, this expression pattern was unaltered in *Abph2* mutants. More careful investigation showed that *Abph2* transcripts start to accumulate in embryos from 12 days after pollination (DAP), but we detected a missexpression of *Abph2* at 18 DAP, when ~4 leaves have been initiated. This altered expression pattern likely explains the altered phenotype in the mutant.

The *Abph2* gene is identical to *MSCA1*. Loss of *MSCA1* leads to male sterility but a normal phyllotaxy. A detailed meristem size comparison revealed a significant decrease in *msca1* mutants. This finding demonstrates that *MSCA1* normally functions to promote meristem growth. *Abph2/MSCA1* encodes a CC-type glutaredoxin protein, similar to *Arabidopsis ROXY1* and *ROXY2* genes. Recently, ROXY1 and *ROXY2* were found to interact with basic leucine zipper (bZIP) transcription factors, including PERIANTHIA (PAN), which is related to the FASCIATED EAR4 (FEA4) recently cloned in our lab. Loss of *FEA4* function results in a larger meristem, as well as altered phyllotaxy,

similar to *Abph2*. Using yeast two-hybrid and bimolecular fluorescence complementation (BiFC) assays, we confirmed that ABPH2 and FEA4 interact, suggesting that these genes may define a new pathway in meristem proliferation control.

The Regulation of Meristem Size in Maize

B. Il Je, Q. Wu, M. Pautler, T. La Rue, A. Masson, P. Bommert, S. Lee, 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, 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, *fasciated ear2* and *thick-tassel dwarf1*, encode orthologs of the *Arabidopsis thaliana* genes *CLAVATA1* and *CLAVATA2*, indicating 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.

ct2 is a classical mutant of maize that exhibits a fasciated inflorescence phenotype as well as semidwarfism. Using a map-based cloning approach, we found 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. *ct2* is epistatic to *fea2* with respect to spikelet density and meristem size, suggesting that they act in a common pathway. We made a CT2 fusion with YFP driven by its endogenous promoter and found localization to the plasma membrane. In coimmunoprecipitation experiments (Co-IPs), we found that FEA2 and CT2 interact, implicating heterotrimeric G-protein signaling in the CLAVATA signaling pathway. To gain a better understanding of the cross-talk between G-protein signaling and the CLAVATA pathway, we need to determine whether CT2 directly interacts with FEA2 or if other proteins mediate their interaction. Our Co-IP experiments in *Nicotiana benthamiana* suggested that removing the intracellular tail of FEA2 did not affect the FEA2–CT2 interaction, indicating that their interaction may require other mediators. Additionally, our Co-IP results showed that the pseudokinase CORYNE interacts with FEA2, but not CT2, indicating that CORYNE is not the mediator of FEA2 and CT2 interactions. Using immunoprecipitation (IP)-mass spectrometry, we have identified an LRR-RLK protein that interacts with both FEA2 and CT2. This interaction has been further confirmed using Co-IP approaches. Currently, we are trying to figure out if this LRR-RLK is required for FEA2–CT2 interactions. This research introduces a new paradigm in G-protein signaling, because G proteins interact exclusively with seven pass *trans*-membrane receptors in mammals and fungi.

Another fasciated ear mutant that we have cloned is *fasciated ear 3* (*fea3*), which was derived from a radiation mutagenesis screen in Russia. *fea3* shows an overproliferation of the inflorescence meristem. We cloned this 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 from an EMS-targeted mutagenesis. *fea3* encodes a predicted leucine-rich repeat receptor-like protein, related to *fea2*. In situ hybridization and RFP-tagged transgenic plants show that FEA3 is expressed in the organizing center of the SAM, as well as in the root apical meristem (Fig. 4). FEA3 is localized in the plasma membrane and intracellular vesicles. To determine whether FEA3 responds to a CLV3-related (CLE) peptide, we tested its sensitivity to different peptides. *fea3* mutants showed reduced peptide sensitivity, but interestingly, they responded to a different CLE peptide compared to *fea2*. Double mutants of *fea2*/*fea3* and *td1*/*fea3* have additive and synergistic fasciated phenotypes, indicating that they may act in independent pathways that converge on the same downstream target to control meristem size. The CLAVATA receptors restrict WUS expression to the organizing center (OC) and prevent its spread to the overlying central (CZ) and peripheral zone (PZ). However, the mechanism restricting WUS expression from the rib zone (RZ) below is poorly

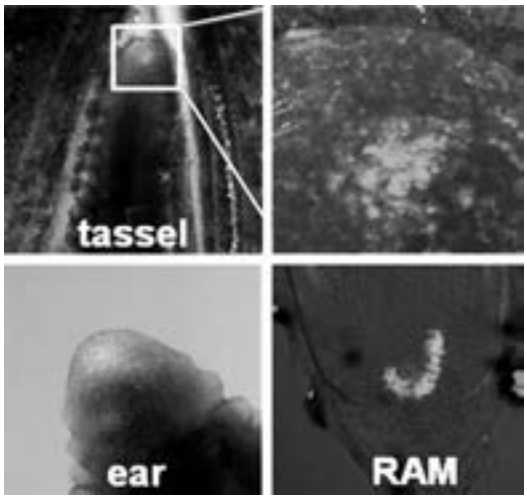

Figure 4. RFP-tagged FEA3 is expressed in the organizing center of inflorescence meristems of ear and tassel and in vascular initial cells of the root apical meristem (RAM).

understood. Comparing FEA3-RFP and WUS-RFP, we find that FEA3 is expressed in the RZ just below the region of WUS expression, and WUS expression spreads downward in *fea3* mutants. *fea3* ortholog mutants in *Arabidopsis* resemble *CLAVATA* mutants in having fasciated inflorescence stems, but they do not show abnormal flower structure like *CLAVATA* mutants. These results indicate that FEA3 is a receptor protein that functions in a new pathway distinct from that of known CLAVATA receptors.

Another mutant, *fasciated ear4*, is a semidwarfed maize mutant that also has fasciated ears and tassels. These phenotypes are caused by enlarged vegetative and inflorescence meristems, which have lost control of the pathways normally regulating meristem size. We mapped *fea4* to a bZIP transcription factor with an EMS-induced base transition causing a premature stop codon. Two additional alleles also contained mutations in the gene, and we obtained a series of transposon-induced mutant alleles through collaboration with DuPont Pioneer, confirming that we have identified a novel gene required for the control of meristem size in maize.

We have subsequently investigated the function of *fea4* through several parallel approaches. We used in situ hybridization to determine its expression pattern during different stages of development. During vegetative development, *fea4* is expressed in the peripheral zone of the SAM and is dramatically excluded from the stem cell niche and the site of leaf initiation. This unique expression pattern suggests that *fea4* does not act by directly influencing the stem cells in the central zone of the SAM, but rather by influencing the process of differentiation in the peripheral zone. We have generated plants expressing a translational fusion of YFP with FEA4 under the control of its native promoter. These plants express the fusion protein in the nucleus of cells in the expected domain, closely matching the mRNA expression pattern. We used these lines for chromatin immunoprecipitation followed by high-throughput sequencing (ChIP-Seq) experiments to determine genome-wide binding of FEA4 to its target loci. The YFP-tagged transgenic lines will also be used for IP-mass spectrometry experiments in the future. We have also obtained a global picture of transcriptional changes in the mutant by mRNA sequencing, and are beginning to follow up on downstream genes that may mediate *fea4* action. A number of genes that showed significant expression differences in *fea4* mutants compared to wild-type siblings were involved in determinacy and differentiation, including key developmental regulators of maize inflorescence architecture, and in auxin-based signaling and transport. The majority of genes in these categories were down-regulated in the *fea4* mutant, suggesting that FEA4 promotes their expression, whereas genes that were up-regulated in the mutant tended to be related to metabolic processes. 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.

Beyond these advances, we are in the process of mapping additional fasciated ear mutants from EMS mutagenesis screens. We have determined approximate positions for several of these mutants and are proceeding with fine mapping and molecular cloning.

Defining the Regulatory Networks Controlling Inflorescence Architecture in Maize

A.L. Eveland, M. Pautler, T. La Rue, A. Goldshmidt [in collaboration with D. Ware and S. Kumari, CSHL/USDA-ARS; S. Hake and M. Lewis, PGEC/USDA-ARS, University of California, Berkeley; E. Vollbrecht, Iowa 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 agronomic importance in determining yield. Inflorescence architecture among cereal crops is diverse, yet characterized by a unique morphology, where flowers are borne on short, determinate branches called spikelets. In maize, these spikelets are paired, an exclusive feature of the tribe Andropogoneae, which includes other important cereal and bioenergy crops. Variations in inflorescence branching patterns arise from position and developmental fate of differentiating stem-cell populations called meristems. The coordinated actions of key developmental regulators, as well as genetic interactions between them, modulate meristem initiation, size, and determinacy during maize inflorescence development. Our goal is to understand the mechanisms by which these regulators act to control inflorescence architecture, what other factors and/or biological processes are involved in this regulation, and how these regulatory networks coordinate development in space and time.

Our approach integrates large-scale genomics data sets, such as gene expression profiles achieved by high-throughput RNA sequencing, with discrete morphological features in development. Here, we characterized precise timing of developmental transitions at the molecular level in maize inflorescences, by associating spatiotemporal dynamics in gene expression with morphological changes resulting from genetic perturbations. For the latter, we used loss-of-function mutants in three important regulators of the *ramosa* (*ra*) pathway, which controls stem-cell fate and the decision to branch.

In a recently published study (Eveland et al. 2014), we identified discrete developmental modules that contribute to identity and determinacy of grass-specific meristem types. Notably, we defined a module for spikelet pair meristem determinacy consisting of coexpressed genes that were largely misexpressed in *ra1* mutants, including known determinacy factors coopted from other developmental contexts, along with genes of unknown function. In situ hybridizations for candidate genes in this module showed that they shared largely overlapping and/or adjacent expression domains during development. To elucidate additional factors that may act upstream in the control of this determinacy module, we mined for enrichment of known *cis*-regulatory elements within the promoters of coexpressed genes. We identified experimentally defined binding sites for bZIP transcription factors (TFs) and KNOTTED1 among the most highly enriched regulatory elements associated with this module. Consistent with this finding, many of the coexpressed genes within this module were identified as putative targets of KN1 and FEA4, a bZIP TF, based on ChIP-Seq profiles. We are currently integrating TF occupancy maps from different ChIP-Seq experiments, and by associating cobound targets with RNA-Seq-based transcript profiles from respective loss-of-function mutants, we are beginning to elucidate combinatorial gene regulation on a genome-wide scale. For example, we found that ~50% of the high-confidence FEA4-binding sites overlapped directly with those of KN1. Differential expression analysis of genes proximal to shared binding sites suggested that KN1 and FEA4 acted either together or in opposition to regulate a number of genes involved in organ differentiation and auxin-related processes, suggesting interfaces between meristem maintenance and size pathways. FEA4 also bound and modulated *ramosa1*, indicating a link between meristem size and determinacy pathways. Furthermore, *fea4* gene expression is significantly down-regulated in *ra1*, *ra2,* and *ra3* loss-of-function mutants, suggesting feed-forward regulation.

Our RA1 ChIP-Seq experiment identified ~1000 high-confidence target genes in proximity to sites bound by RA1. Of these, approximately one-fourth showed altered expression in the *ra1* mutant background, suggesting that they are modulated targets of RA1. We also showed that RA1 acts as both an activator and a repressor of gene expression, and the mechanism of RA1 action is dependent on spatiotemporal context. One of the more interesting RA1 target genes is *liguleless1* (*lg1*), which appears to be directly repressed by RA1 in determinate spikelet pair meristems. We further showed that in the absence of RA1, *lg1* is expressed at the base of long inflorescence branches, for example, in *ra1* mutant ears and long tassel branches. This finding is consistent with recent work that identified *lg1* as a key candidate locus in an association study for inflorescence architecture traits. One hypothesis is that *lg1* may be promoting branch identity by initiating a boundary, similar to its vegetative role in specifying the blade-sheath boundary. To test conserved and divergent mechanisms of LG1 function in the leaf versus the inflorescence, we are using ChIP-Seq to identify LG1 targets in both tissue types. We are also further investigating potential regulatory motifs that are enriched within the binding sites of RA1 to resolve consensus TF-binding sites for

RA1 and/or cofactors that may be involved in regulation of its downstream targets.

Functional characterization of candidate genes from these networks is currently under way, including in situ hybridizations, analysis of loss-of-function mutant alleles, and crosses to *ra* mutants to reveal potential genetic interactions. Because *ra1* has been implicated as an important locus in the domestication of maize and is found only in Panicoid grasses, these data are being used in comparative analyses with other grasses to further understand RA1 function and the evolution of grass inflorescence architecture. We are also now trying to understand the effects of environmental perturbation, specifically drought stress, on these developmental networks.

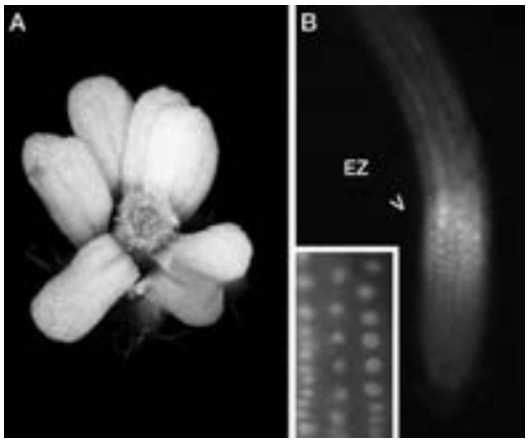

Figure 5. (*A*) *tpp* mutants make extra floral organs. (*B*) A TPP-YFP fusion shows expression in the root elongation zone (EZ) and is nuclear (inset).

Control of Branching and Determinacy in Plant Shoots

Y.G. Wolfenson, T. Zadrozny, R. Willen, S. Hiraga

The *RAMOSA* (*RA*) genes in maize function to impose determinacy on axillary meristem growth, and consequently, *ra* loss-of-function mutants (*ra1, ra2,* and *ra3*) have more highly branched inflorescences. *RA3* encodes a predicted metabolic enzyme, a trehalose phosphate phosphatase. The disaccharide trehalose is not abundant in plant tissues, and it may have a regulatory role because it has been implicated in stress protection, control of sugar signaling, and regulation of photosynthetic rate. Ours 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. The *RAMOSA* (*RA*) genes 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, since it affects the expression of specific genes.

To further investigate the mechanism of RA3 action, we searched for mutants in *Arabidopsis* orthologs. One of them has an interesting phenotype that is related to development and determinacy. Its late-flowering phenotype is accompanied by reduced size and shorter roots and fusion of cauline leaves to the main stem. Furthermore, double mutants with an additional TPP paralog show indeterminate floral phenotype of the *ra3* maize phenotypes. A YFP fusion of one of the TPP genes shows nuclear localization in roots, supporting the idea that it has regulatory functions (Fig. 5B).

Additionally, we are taking genetic approaches to identify factors that act in the same pathway with RA3 to control spikelet pair meristem determinacy, by screening for enhancer/suppressor modifiers of the *ra3* phenotype. Typically, *ra3* mutants in a B73 background have three to eight branches only at the base of the ear; we mutagenized *ra3* mutants and looked for plants that have more branches and/or have branches at the upper part of the ear. So far, we have identified several enhancers and have mapped two of them. One maps to an *ra3* paralog and the second to a giberellic acid metabolic gene, suggesting that GA hormone signaling may interface with branching regulation.

Natural Variation and Inflorescence Architectures

S. Vi, I. Liao, P. Bommert

Maize inflorescence architectures have been a target for extensive selection by breeders since domestication; hence, different maize inbreds vary greatly in these traits. The genetic basis underlying this diversity is

largely unknown and is of great interest for both fundamental and applied science. Now with the wealth of genomic data, we are equipped to study them. To identify natural variation relevant to inflorescence traits, we looked for inbred backgrounds that can enhance/suppress the phenotypes of the mutants on which we work. We focused on the 25 NAM (nested association mapping) founder inbreds because they were selected to capture the diversity of maize germplasm and because of the genetic tools available for these inbreds. We have been crossing these 25 inbreds to our collection of mutants (often in B73 background) and screen the F2 for plants with suppressed/enhanced phenotype compared to the mutant in the original inbred background.

We have identified a suppressor of *ramosa3* (*ra3*) coming from the Mo17 inbred, an enhancer coming from the Ki11 inbred, and an enhancer of *fea2* coming from the NC350 inbred. Segregation ratios suggest one semidominant locus for the Mo17-derived suppressor, two or more loci for the Ki11-derived enhancer, and one recessive locus for NC350-derived enhancer. We are now in the process of rough mapping by bulked segregant analysis. Additionally, because the natural modifiers are often results of quantitative trait loci (QTL) rather than single-gene effects, we crossed the mutants to the corresponding NAM-founder/B73 RILs, in order to identify and map potential modifying QTL.

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Huyen Bui and Rachappa Balkunde

PLANT DEVELOPMENTAL BIOLOGY, STEM CELLS, AND FLORAL BRANCHING SYSTEMS

Z.B. Lippman C. Brooks, K. Jiang, K. Liberatore, C. MacAlister, S.J. Park, S. Thomain, C. Xu

Developmental Origins of Plant Shoot Architecture

In plants, populations of stem cells called apical meristems (AMs) give rise to all above-ground organs and guide shoot architecture. After a seed germinates, an AM from the embryo produces a primary shoot with lateral leaves that give rise to AMs in their axils. AMs formed early in a plant's life are initially small and flat and maintain a relatively constant number of cells, enabling persistent growth. Upon perceiving environmental cues that stimulate genetically encoded signals, AMs undergo reproductive transitions, marked by increasing size and becoming a dome. During this transition, leaf shape and size can be altered, internode length can change, and apical dominance is released to promote branching.

Although useful for understanding principles of meristem activity and potential, this framework fails to explain the remarkable architectural diversity of plants, especially variation in the number and arrangement of branches (Park et al. 2014). Branching variation traces back to differences in when and where meristems form, whether they begin growing immediately or experience dormancy, how long they grow, how large they become, and the number of additional meristems they generate. At the center of this diversity lie two processes: (1) meristem maturation, during which AMs repeatedly experience a reduction of a vegetative promoting program and an increase of a floral promoting program, and (2) meristem maintenance, which is responsible for controlling stem-cell proliferation and meristem size. Our research aims to elucidate and understand the mechanisms controlling meristem maturation and maintenance, focusing on the floral branching systems (inflorescences) of tomato and related Solanaceae (e.g., pepper and tobacco). Using the virtues of Solanaceae development, including readily accessible meristems for molecular analysis, we are addressing the hypothesis that diversity in shoot and inflorescence architecture is determined by evolutionary differences in rates of meristem maturation and meristem size.

Tomato Architecture and Flower Production

Tomato plants generate hundreds of multiflowered inflorescences due to sympodial growth, the defining feature of which is that each meristem terminates in a flower and new growth originates from axillary meristems that also terminate, but at varying rates of maturation to control shoot architecture (Fig. 1). In tomato, the primary shoot meristem (PSM) matures gradually to typically produce eight leaves before

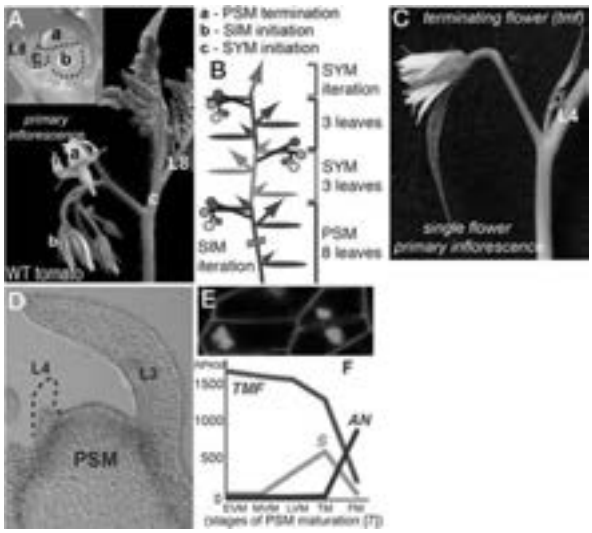

Figure 1. Tomato meristems and sympodial plant architecture in normal and *tmf* mutant plants. (*A–C*) Wild-type shoot architecture (*A,B*) and *tmf* mutant phenotypes (*C*). (*D–F*) *TMF* expression and localization in situ showing expression at periphery of vegetative meristem (*D*), 35S:TMF-GFP leaves showing localization in nuclei (*E*). RNA-Seq showing expression is highest in vegetative meristems (*F*).

terminating in the first flower of the primary inflorescence. This gradual maturation leads the PSM into a full reproductive state, which is required to initiate two types of axillary "sympodial" meristems. Just below the terminating PSM, a sympodial inflorescence meristem (SIM) develops and gives rise to one new SIM before quickly terminating with no intervening leaves. Several iterations of this process produce a compound multiflowered inflorescence composed of fused short floral branches. Later inflorescences likewise originate from sympodial meristems; in the axil of the last PSM leaf, a sympodial vegetative meristem (SYM) matures at an intermediate rate to develop three leaves before terminating in the first flower of the second inflorescence. This process repeats to produce a compound vegetative shoot with equally spaced inflorescences. Axillary meristems from lower leaves repeat the PSM. Our research aims to elucidate the mechanisms controlling meristem maturation and maintenance in order to gain an understanding of the diversity of Solanaceae inflorescences ranging from single flowers (e.g., pepper) to highly branched with dozens of flowers (e.g., wild tomatoes).

The Role of TERMINATING FLOWER in Inflorescence Architecture

C. MacAlister, C. Xu

Our prior work has shown that mutations in *COMPOUND INFLORESCENCE* (*S*, homolog of *WUSCHEL HOMEOBOX 9*), *ANANTHA* (*AN,* homolog of *UNUSUAL FLORAL ORGANS*), and *FALSIFLORA* (*FA*, homolog of *LEAFY*) cause more SIMs, and thus branches and flowers, to develop, because termination is delayed (*s* mutants) or never achieved (*an*, *fa*) (Jiang et al. 2013). *S* is expressed late in maturation just before activation of *AN*, which encodes an F-box protein that interacts with its transcription factor partner FA to form a floral specification complex. Thus, one mechanism promoting maturation and multiflowered inflorescences involves *S* leading meristems to a reproductive stage when *AN* can activate to trigger floral termination, which, in turn, is required for the initiation and proper maturation of SIMs. Given a mechanism promoting maturation, we wondered whether a mechanism also exists to repress maturation, or maintain a vegetative meristem state. Compromising such a mechanism might lead to more rapid termination and inflorescences with fewer flowers. We therefore turned to the *tmf* mutant, which flowers early and produces a single flower inflorescence on the primary shoot (Fig. 1). We found that *TMF* encodes a member of the ALOG (Arabidopsis LSH1 and Oryza G1) protein family, of which there are 12 members in tomato. *TMF* expression peaks in vegetative meristems in a boundary domain, then drops when *AN* is activated. ALOGs contain a DNA-binding domain, suggesting transcription factor function; indeed, TMF is in the nucleus and can activate transcription in yeast, but native targets are unknown. In a yeast two-hybrid (Y2H) screen using TMF as bait, we identified several transcriptional regulators, the most prominent being homologs of the *Arabidopsis* BTB/POZ domain-containing BOP1 and BOP2 proteins, which are expressed in boundary domains like *TMF* and are best known for roles in leaf development, floral patterning, and organ abscission. Tomato has three *BOP* genes (*tBOP1/2/3*), and based on bimolecular fluorescence complementation (BiFC) confirming all three tBOP proteins interact with TMF in tomato nuclei, we have begun generating RNA interference (RNAi) knockdown (*tbop-kd*) plants. Phenotypic and molecular analyses of our first *tbop-kd* plants suggest that *tBOP* genes function with *TMF* to control inflorescence architecture by preventing precocious activation of *AN* transcription during the vegetative phase of meristem growth. We are now developing resources to identify transcriptional targets of *TMF* and will also perform detailed developmental and molecular analysis of *tBOP* genes to understand their roles in flowering and other aspects of tomato development.

Divergent Evolution of Genes and Gene Expression Programs Underlies Solanaceae Inflorescence Diversity

K. Jiang, S.J. Park

Evolution of phenotypic diversity traces to divergent evolution of genes and proteins by changes in the regulatory networks that lead to differential execution of conserved developmental programs. One hypothesis for the range of diverse inflorescence forms in the Solanaceae family ranging from a single flower to highly branched multiflower inflorescences is that meristems mature at different rates to guide final inflorescence form. To test this idea and investigate if and to what extent there is evolutionary variation in rates of

maturation, we used deep sequencing to capture transcriptome dynamics of meristem maturation from five Solanaceae species representing the range of inflorescence architecture. Transcriptomes across species are more similar at both early (vegetative) and late (floral) meristem maturation stages, but more divergent at the intermediate stage when the transition from vegetative to reproductive growth occurs. Interestingly, genes that peak in expression in early and late stages are evolutionarily more conserved at the protein level compared to genes that peak during the transition. By comparing expression dynamics of orthologous genes, we established a Solanaceae Transcriptome Maturation Index (TMI), defined by gene expression weighted by their "earliness" in expression during meristem maturation. Using the TMI, we found that greater branching is associated with a pronounced delay in meristem maturation compared to tomato, whereas production of simple inflorescences, such as the single flower inflorescences of pepper, is associated with a more rapid maturation. Our work has led us to propose a "bubble" model of Solanaceae meristem maturation, in which greater divergence in gene expression dynamics and protein similarities during the reproductive transition compared to vegetative and floral stages is responsible for modulating inflorescence diversity.

Inflorescence Architecture and the Control of Meristem Size

K. Liberatore, C. MacAlister, S. Thomain

In many plants, inflorescence branching is influenced by meristem size. Meristem size is tightly controlled through a process of "meristem maintenance," in which stem cells lost to lateral organ formation are continuously replenished through cell division. Work in *Arabidopsis,* maize, and rice has shown that conserved components of the *CLAVATA* (*CLV*) signaling pathway control meristem maintenance by restricting stem cell proliferation, but how meristem size is regulated in other plants remains poorly understood. By studying tomato mutants showing "fasciated" phenotypes of inflorescence branching and enlarged flowers and fruits, we isolated a mutation in *FASCIATED AND BRANCHED* (*FAB*, ortholog of the *Arabidopsis* receptor kinase gene *CLV1*), indicating that the *CLV* pathway is conserved in tomato (Fig. 2). We also discovered *fasciated inflorescence* (*fin*) and *fasciated and necrotic* (*fan*), both of which are defective in genes encoding glycosyltransferases (GTs), specifically a hydroxyproline *O*-arabinosyltransferase and arabinosyltransferase, respectively. Arabinosyltransferases are enzymes that transfer arabinoside moieties to diverse

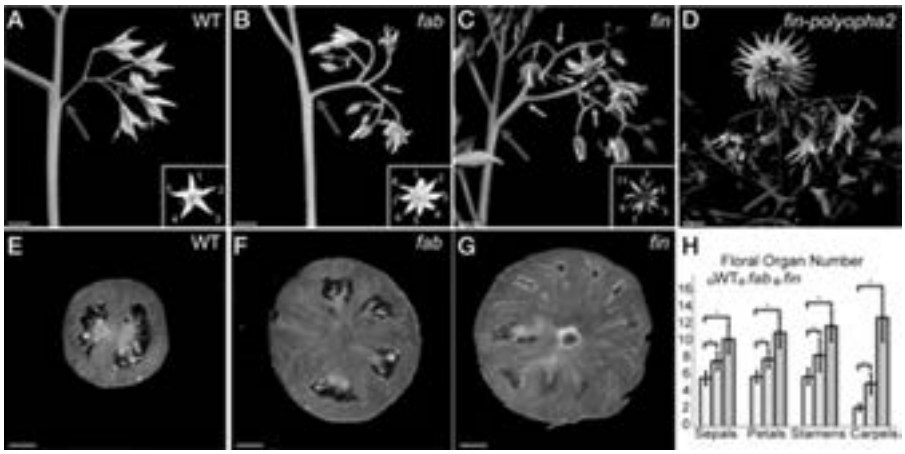

Figure 2. *fin* and *fab* mutant phenotypes. (*A–H*) Images and quantification of mature inflorescence (IF), flower, and fruit phenotypes. Representative secondary inflorescences of wild type (*A*), *fab* (*B*), and *fin* (*C*) and the primary IF of *fin* (*D*). (*D*) The *fin-polyopha2* mutant from *Solanum pimpinellifolium.* Floral image insets within each panel show the increased number of sepals and petals in both *fab* and *fin* mutants. The base of each IF is marked with an arrow, and points of IF branching are marked with arrows; bars, 1 cm. (E–G) Mature fruits showing increased locule number and fruit size in *fab* and *fin* mutants that converts the wild-type M82 roma-sized tomatoes to beefsteak-sized; bars, 1 cm. (*H*) Quantification of floral organ number (mean ± S.D.).

proteins, including a three-arabinoside chain on the small signaling peptide CLV3 whose biological relevance has been unclear. Thus, our discovery of FIN and FAN suggests that GTs have critical roles in stem-cell proliferation and that glycosylation of tomato CLV3 might be required for activity. Interestingly, *fan* mutants also senesce prematurely, leading to necrosis and death, which might result from a failure to properly glycosylate other important signaling peptides, such as PSY1, recently shown in *Arabidopsis* to regulate hormonal balance and defense. We are now aiming to integrate genetic, molecular, and biochemical approaches to study FIN and FAN in order to understand the roles of glycosylation in the signaling pathways that regulate stem-cell proliferation, senescence, and defense signaling in tomato.

Diverse Developmental Roles for the FIN Family

C. MacAlister

FIN is a member of a deeply conserved GT family. To compare developmental roles of *FIN* genes in diverse species, we analyzed mutations in the three *FIN-like* family members in *Arabidopsis*. Surprisingly, no mutant phenotypes were observed in the single mutants, but double mutants of *finl1;finl2* showed severe pollen tube growth defects. Although double-mutant pollen can germinate, pollen tubes arrest in the transmitting tract of the ovary and fail to target ovules. Such striking phenotypic consequences between mutations in tomato and *Arabidopsis FIN* genes is surprising considering the deep conservation of this GT family. To further explore *FIN* family function, we created mutations in two paralogous *FIN* genes in the moss *Physcomitrella patens*. Moss development involves germination of a haploid spore followed by formation of a vegetative body (colony) composed of filamentous protonemal network containing two cell types: slower-growing chloronema for photosynthesis and faster-growing caulonema to colonize the surrounding substrate. Interestingly, knocking out the more highly expressed *FIN* gene (*PpFINLa*) results in larger and faster colonizing colonies, owing to enhanced caulonema production relative to chloronema. Importantly, although pollen tubes and caulonema are both tip-growing cells, the process of tip growth itself is unaffected in both systems. These results point to an exciting link between glycosylation and meristem size regulation, pollen tube guidance, and the balance between filament types in moss. We are currently investigating whether FIN proteins target distinct sets of proteins for glycosylation in different systems and developmental contexts.

Improving Flower Production and Yield through Dosage Sensitivity of the Florigen Pathway

S.J. Park, K. Jiang, K. Liberatore

For more than a century, it has been known that inbreeding harms plant and animal fitness, whereas interbreeding between genetically distinct individuals can lead to more robust offspring in a phenomenon known as heterosis.

Although heterosis has been harnessed to boost agricultural productivity, its causes are not understood. Especially controversial is a model called "overdominance," which states in its simplest form that a single gene can drive heterosis. In tomato, a mutation in just one of two copies of the gene *SINGLE FLOWER TRUSS* (*SFT*) encoding the flowering hormone called florigen causes remarkable increases in yield, but it is not known why. One clue is that overdominance is only observed in the background of "determinate" plants, in which the continuous production of side shoots and inflorescences gradually halts due to a defect in the flowering repressor *SELF PRUNING* (*SP*). One possibility we have explored is that *sft* overdominance is based on epistatic interactions between *SFT* and *SP*, which have opposing roles in flowering time and shoot architecture. Using transcriptome sequencing of shoot meristems, we have found that *sft* mutant heterozygosity causes weak semidominant delays in flowering of both primary and side shoots, which extends the period of flower and fruit production. Thus, *sft* heterozygosity triggers a yield improvement by optimizing plant architecture via its dosage response in the florigen pathway.

Our findings suggest that dosage sensitivity of florigen and potentially other genes in the florigen pathway could be exploited to further improve tomato architecture and yield. Given that heterozygous mutations in *SFT* partially relieve *sp*-imposed determinacy, we screened for new suppressors of determinacy and isolated a weak allele of *sft* and two

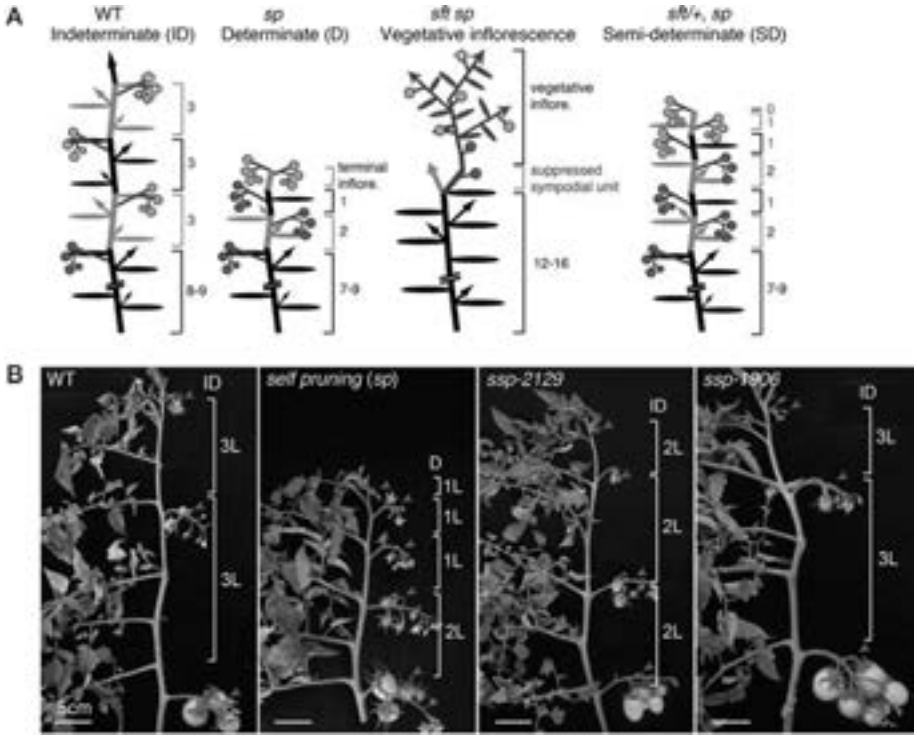

Figure 3. Mutations that suppress *sp*. (*A*) Diagrams show left-to-right architectures of wild-type, *sp* mutants, *sft sp* double mutants, and *sft*/+ *sp* plants. The latter two genotypes suppress the *sp* determinate phenotype, and *sft*/+ mutant heterozygosity leads to increased flower production and yield. (*B*) Images show new *suppressor of sp* (*ssp*) mutants that restore indeterminacy and result in a desirable form of sympodial growth, in which inflorescences form every two leaves instead of every three (*ssp-2129*).

mutations in *SUPPRESSOR OF SP* (*SSP*), the latter resulting in a highly desirable indeterminate form in which flower clusters (inflorescences) continuously develop every two leaves instead of the typical three (Fig. 3). We show that the *SSP* mutations disrupt a critical motif in a bZIP transcription factor required to establish a "florigen activation complex." By generating all combinations of *sft* and *ssp* mutant heterozygotes in the *sp* background, we created a quantitative range of determinate architectures and found that specific combinations of *ssp* and *sft* double heterozygotes provide a new architectural optimum that leads to the highest yields. Our findings show the power of using florigen pathway mutations to customize tomato architecture, flower production, and yield, and they suggest that creating similar genetic toolkits in other crops could broadly benefit future breeding.

PUBLICATION

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In Press

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EPIGENETIC INHERITANCE IN PLANTS AND FISSION YEAST

R. Martienssen
S. Bhattacharjee
S. Castel
A.-Y. Chang
K. Creasey
F. de Sousa Borges
M. Donoghue
E. Ernst
J.-J. Han
R. Herridge
Y. Jacob
H.S. Kim
C. LeBlanc
S.C. Lee
A. Molla-Morales
J.-S. Parent
A.C. Pastor
M. Regulski
J. Ren
B. Roche
A. Schorn
A. Shimada
J. Simorowski
R. Tirado-Magallanes
U. Umamaheswari
C. Underwood

Plants and fission yeast provide excellent model organisms to address the question of how epigenetic information is propagated, including transposon regulation, heterochromatic silencing, and gene imprinting, important both for plant breeding and for human health. We are investigating the role of RNA interference (RNAi) in heterochromatic silencing in the fission yeast *Schizosaccharomyces pombe* and in the plant *Arabidopsis thaliana*, as well as continuing our work on *Zea mays*. In fission yeast, we have found additional evidence that DNA replication promotes spreading of heterochromatin and that RNAi promotes replication by releasing RNA polymerase II. In plants, we have found that the genome undergoes limited reprogramming of DNA methylation in pollen, guided by DNA glycosylases and small RNA. Using mutants deficient in histone and DNA methylation, we have found that histone variants and microRNA (miRNA) contribute to reprogramming by targeting transposons. Our results suggest a model for imprinting, transposon control, and the origin of epialleles. We continue to develop duckweeds as a source of biofuel and have developed an efficient transformation and gene control system in *Lemna minor*. Sequencing of the oil palm genome has provided some important clues as to how to increase oil yields and reduce tropical deforestation.

This year, we said good-bye to Fred vanEx, Joe Calarco, and Alex Canto Pastor, who left for positions in Gent, Stanford, and Cambridge, respectively. We welcomed postdocs Rowan Herridge (University of Otago), Sonali Bhattacharjee (Oxford University), and Jean Sebastien Parent (INRA Versailles, Paris) and student Roberto Tirado-Magallanes (UNAM, Mexico).

Reprogramming the Epigenome in *Arabidopsis* Pollen

F. Borges, J.P. Calarco, Y. Jacob, C. LeBlanc, F. Van Ex
[in collaboration with F. Berger, Temasek Life Sciences Laboratory Singapore; T. Kenoshita, Nara Institute of Science, Japan; T. Higashiyama, Nagoya University, Japan]

Germline reprogramming of DNA methylation is important for transposon silencing and epigenetic inheritance. In plants, the male gametophyte is derived from haploid microspores via two postmeiotic cell divisions to give rise to the gametes (sperm cells, SC) and the vegetative cell (VC). The purification of these three individual cell types, coupled with genome-wide DNA methylation analysis and small RNA sequencing, has revealed a dynamic regulation of the epigenome during gametogenesis. In *Arabidopsis*, the frequency of single-base variation of DNA methylation is much higher than genetic mutation, and, interestingly, variable epialleles are premethylated in the male germline. However, these same alleles are targeted for demethylation in the pollen vegetative nucleus, by a mechanism that seems to contribute to the accumulation of small RNAs that reinforce transcriptional gene silencing in the gametes. In mammals and plants, parental genomic imprinting restricts the expression of specific loci to one parental allele. Interestingly, imprinted loci are also demethylated in the vegetative nucleus (VN) resembling variable epialleles in accumulating small RNA in sperm. We have found that

de novo RNA-directed DNA methylation (RdDM) regulates imprinting at some of these loci when they are expressed in the endosperm. RdDM in somatic tissues is required to silence expression of the paternal allele. In contrast, the repression of RdDM in female gametes participates with or without the DME requirement in the activation of the maternal allele. The contrasted activity of DNA methylation between male and female gametes appears to be sufficient to prime imprinted maternal expression. After fertilization, MET1 maintains differential expression between the parental alleles. RdDM depends on small interfering RNAs (siRNAs). The involvement of RdDM in imprinting supports the idea that sources of siRNAs such as transposons and de novo DNA methylation were recruited in a convergent manner in plants and mammals in the evolutionary process leading to selection of imprinted loci.

Selective Methylation of Histone H3 Variant H3.1 Regulates Heterochromatin Replication

Y. Jacob, M.T.A. Donoghue, C. LeBlanc, C.J. Underwood, [in collaboration with S. Michaels, Indiana University; D. Reinberg, New York University Medical School; and J.-F. Couture, University of Ottawa]

Histone variants have been proposed to act as determinants for posttranslational modifications (PTM) with widespread regulatory functions. We have found a histone-modifying enzyme that selectively methylates the replication-dependent histone H3 variant H3.1. The crystal structure of the SET domain of the histone H3 lysine 27 (H3K27) methyltransferase ARABIDOPSIS TRITHORAX-RELATED PROTEIN 5 (ATXR5) in complex with an H3.1 peptide shows that ATXR5 contains a bipartite catalytic domain that specifically "reads" alanine 31 of H3.1. Variation at position 31 between H3.1 and replication-independent H3.3 is conserved in plants and animals, and threonine 31 in H3.3 is responsible for inhibiting the activity of ATXR5 and its paralog ATXR6 (Fig. 1). Our results suggest a simple model for the mitotic inheritance of the heterochromatic mark H3K27me1 and the protection of H3.3-enriched genes against heterochromatization during DNA replication. Unmodified histone H3.1 may trigger overreplication of heterochromatin and contribute to reprogramming in pollen that lacks H3.1 in the VN.

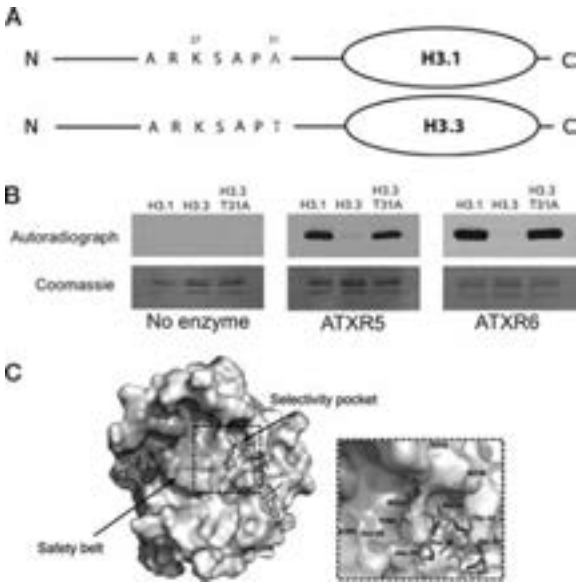

Figure 1. The selectivity pocket and safety belt of ATXR5/6-type H3K27 methyltransferases are responsible for histone H3.1 preference over histone H3.3. (*A*) Alignment of the amino-terminal tails of the canonical histone H3 variants H3.1 and H3.3 from *A. thaliana* highlighting residue 31A or T. (*B*) In vitro histone lysine methyltransferase assay using recombinant chromatin containing plant histone H3.1, plant histone H3.3 or plant histone H3.3 T31A. (*C*) The structure of the ATXR5-H3.1-SAH complex in electrostatic potential surface representation with the selectivity pocket and safety belt highlighted. Positive and negative potentials are in gray and dark gray, respectively. (Inset) Enlarged view of the residues forming the surface of the selectivity pocket (three-letter code refers to histone H3.1 residues; one-letter code refers to RcATXR5). Hydrogen bonds are shown as dashed red lines.

miRNAs Trigger Widespread Epigenetically Activated siRNAs from Transposons in *Arabidopsis*

K.M. Creasey, M. Regulski, F. Borges, F. Van Ex [in collaboration with B.C. Meyers, University of Delaware]

Transposons in *Arabidopsis* give rise to abundant 21-nucleotide "epigenetically activated" small interfering RNAs (easiRNAs) in *DECREASE IN DNA METHYLATION1* (*ddm1*) and *DNA METHYLTRANSFERASE1 (met1*) mutants, as well as in pollen from wild-type plants, in which heterochromatin is lost during reprogramming. easiRNA biogenesis is dependent on *ARGONAUTE1* (*AGO1)*, *DICER-LIKE4 (DCL4),* and *RNA-DEPENDENT RNA POLYMERASE6 (RDR6*), resembling 21-nucleotide *trans*-acting (ta)siRNAs and other secondary siRNA in this respect. However, the factor that triggers easiRNA biogenesis from transposons

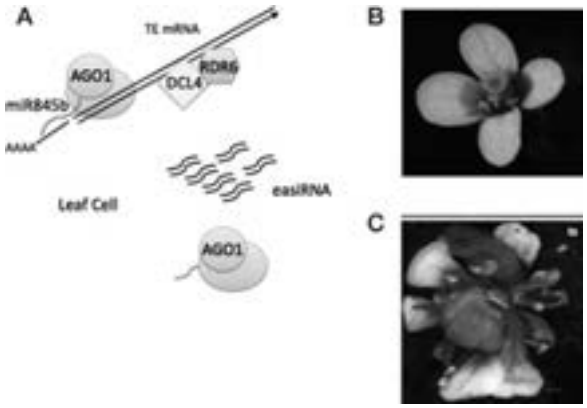

Figure 2. microRNA (miRNA) triggers epigenetically activated small interfering RNA (easiRNA) from transposons in *Arabidopsis*. miRNA such as miR845, which is expressed in pollen, targets transposon transcripts for cleavage and processing by Argonaute 1 (AGO1), RDR6, and DCl4 to generate 21-nucleotide easiRNA (*A*). This process occurs in *decrease in DNA methylation 1 (ddm1)* mutants, which lose transcriptional silencing but resemble wild-type plants (*B*). Failure to generate easiRNA in *ddm1rdr6* double mutants results in severe epigenetic defects and infertility (*C*).

has remained elusive. We have found that specificity is provided by targeted cleavage of thousands of transposon transcripts by at least 50 miRNAs, some of which are themselves encoded by transposons, but most of which are highly conserved and have well-known roles in plant development (Fig. 2). Interestingly, the loss of easiRNAs in *ddm1 rdr6* is compensated by the gain of 24-nucleotide heterochromatic (het)siRNAs that can guide RNA-directed DNA methylation (RdDM). This suggests that *RDR6*-directed easiRNA production acts antagonistically to *RDR2*-directed hetsiRNA production, thereby inhibiting transcriptional gene silencing. Widespread targeting of transposons may reflect the evolutionary origin of miRNA in genome surveillance.

The Maize Methylome Influences mRNA Splice Sites and Reveals Widespread Paramutation-Like Switches Guided by Small RNA

M. Regulski, M.T. Donoghue [in collaboration with W.R. McCombie, J. Hicks, and D. Ware, Cold Spring Harbor Laboratory; J. Reinders, S. Tingey, and A. Rafalski, DuPont Pioneer; A. Smith, University of Southern California, Los Angeles]

The maize genome, with its large complement of transposons and repeats, is a paradigm for the study of epigenetic mechanisms such as paramutation and imprinting. We have determined the genome-wide map of cytosine methylation for two maize inbred lines, B73 and Mo17. CG (65%) and CHG (50%) methylation (where H = A, C, or T) is highest in transposons, whereas CHH (5%) methylation is likely guided by 24-nucleotide, but not 21-nucleotide, siRNAs. Correlations with methylation patterns suggest that CG methylation in exons (8%) may deter insertion of Mutator transposon insertion, whereas CHG methylation at splice acceptor sites may inhibit RNA splicing. Using the methylation map as a guide, we used low-coverage sequencing to show that parental methylation differences are inherited by recombinant inbred lines. However, frequent methylation switches, guided by siRNA, persist for up to eight generations, suggesting that epigenetic inheritance resembling paramutation is much more common than previously supposed. The methylation map will provide an invaluable resource for epigenetic studies in maize.

Tunicate 1 and the Origin of PodCorn

J.-J. Han, M. Regulski [in collaboration with D. Jackson, Cold Spring Harbor Laboratory and the National Park Service, Navajo]

Podcorn was once regarded as ancestral to cultivated maize, and it was prized by pre-Columbian cultures for its magical properties. *Tunicate1* (*Tu1*) is a dominant podcorn mutation in which kernels are completely enclosed in leaf-like glumes. We reported last year that *Tu1* encodes a MADS-box gene expressed in leaves whose 5′ regulatory region is fused by a 1.8-Mb chromosomal inversion to the 3′ region of a gene expressed in the inflorescence. Both genes are further duplicated, accounting for classical derivative alleles isolated by recombination. In young ear primordia, TU1 proteins are nuclearly localized in specific cells at the base of spikelet pair meristems. *Tu1* branch determination defects resemble those in *ramosa* mutants, which encode regulatory proteins expressed in these same cells, accounting for synergism in double mutants. In an effort to trace the origin of podcorn in North America, we are sequencing the genome from samples of ancient corn found at the Betatakin Pueblo in Northern Arizona, which were reported to display the podcorn phenotype.

Large-Scale Identification of Sequence-Indexed *Mu* Insertion Sites in the Maize-Targeted Mutagenesis MTM Population

J.-J. Han, M. Regulski [in collaboration with D. Ware, Cold Spring Harbor Laboratory; P. Ferreira, University of Rio de Janeiro; B. Meeley, Pioneer Hi-bred International]

In maize, the Robertson's *Mutator* (*Mu*) multicopy transposon family has been selected for saturation mutagenesis to produce myriad novel mutant alleles in the maize-targeted mutagenesis (MTM) collection. We have sequenced insertions in MTM lines and found that the position of newly transmitted germinal insertions in the genome can be identified on a large scale. More than 50,000 target sites were detected over all 10 maize chromosomes distributed into hypomethylated genic regions. We detected two parental insertions for each germinal insertion in each plant, which is somewhat higher than expected and likely includes Pack-MULEs that are preferentially located upstream from 5′ termini of genes. We sequenced 96 DNA pools from one grid (48 × 48) containing 2304 plants, and further bioinformatic analysis will create a sequence-indexed MTM *Mu* FST database that will contain up to 100,000 *Mu* insertion sites.

Duckweeds: Genetic Study for Biofuel Production

E. Ernst, A. Molla-Morales, A. Canto Pastor, S.C. Lee [in collaboration with J. Shanklin and J. Schwender, Brookhaven National Laboratories; D. Pappin, Cold Spring Harbor Laboratory; Blake Meyers, University of Delaware]

Petroleum availability and atmospheric carbon accumulation are among the main concerns of our era. Currently, biofuels produced from corn grain and sugarcane are predominant alternatives, but they directly compete for land with food production, raising sustainability concerns. *Lemnaceae* species (aquatic duckweeds) include the smallest flowering plants, and they have considerable potential as biofuel feed stocks because of their extreme growth rates and clonal propagation. Our goal is to engineer duckweeds to increase their oil levels for biofuel production. In order to do so, we aim to increase the expression of genes related to the production of TAG, silence the genes that have a role in the oxidation of lipid bodies, or redirect the starch metabolism to oil production by silencing the key genes that lead to starch accumulation. We have sequenced the genome of *Lemna gibba* DWC131, and gene annotation is currently under way. We have also developed a fast and efficient method for producing stable transgenic fronds in *Lemna minor* via agrobacterium-mediated transformation and regeneration from tissue culture. Additionally, we engineered an artificial miRNA (amiRNA) gene silencing system. We identified the endogenous *Lemna* miR166 precursor and used it as a backbone to produce amiRNAs. As a proof of concept, we induced the silencing of *CH42*, a magnesium chelatase subunit, using our amiRNA platform. These techniques will enable tackling future challenges in the biology and biotechnology of *Lemnaceae*. For example, nitrogen deficiency is an environmental stress that can enhance biosynthesis and storage of starch and triacylglycerol (TAG) in vegetative tissues of plant species such as *A. thaliana* and *Chlamydomonas reinhardtii*, and TAG is a precursor of biodiesel. Based on transmission electron microscopy, we have found that *L. gibba* plants grown on nitrate-lacking media increased the numbers of lipid droplets in the chloroplast, and liquid chromatography–mass spectrometry identified increases in saturated and monounsaturated fatty acids such as palmitic acid (16:0) and palmitoleic acid (16:10). We are currently using our transformation system to enhance oil yields further.

The Oil Palm *Shell* Gene Controls Oil Yield and Encodes a Homolog of SEEDSTICK

R. Martienssen [in collaboration with R. Singh, M. Ong-Abdullah, E.L. Low, R. Nookiah, and R. Sambanthamurthi, Malaysian Palm Oil Board, Kuala Lumpur, Malaysia; E. Lee and R. Desalle, American Museum of Natural History, New York; J. Ordway, S. Smith, M. Budiman, and N. Lakey, Orion Genomics LLC, St. Louis Missouri]

A key event in the domestication and breeding of the oil palm, *Elaeis guineensis*, was loss of the thick coconut-like shell surrounding the kernel. Modern *E. guineensis* has three fruit forms: *dura* (thick-shelled), *pisifera* (shell-less), and *tenera* (thin-shelled), a hybrid between *dura* and *pisifera*. The *pisifera* palm is usually female-sterile, but the *tenera* yields far more oil than

dura and is the basis for commercial palm oil production in all of Southeast Asia. Here, we describe the mapping and identification of the *Shell* gene responsible for the different fruit forms. Using homozygosity mapping by sequencing, we found two independent mutations in the DNA-binding domain of a homolog of the MADS-box gene *SEEDSTICK (STK)*, which controls ovule identity and seed development in *Arabidopsis*. The *Shell* gene is responsible for the *tenera* phenotype in both cultivated and wild palms from sub-Saharan Africa, and our findings provide a genetic explanation for the single-gene heterosis attributed to *Shell,* via heterodimerization. This gene mutation explains the single most important economic trait in oil palm and has implications for the competing interests of global edible oil production, biofuels, and rainforest conservation.

RNAi Promotes Heterochromatic Silencing through Replication-Coupled Release of RNA Polymerase II

J. Ren, S. Castel, A.-Y. Chang, R. Martienssen [in collaboration with W.Z. Cande, University of California, Berkeley; F. Antequera, Universidad de Salamanca, Spain; B. Arcangioli, Institut Pasteur, Paris]

Heterochromatin comprises tightly compacted repetitive regions of eukaryotic chromosomes and has widespread roles in chromosome integrity, stability, and silencing. The inheritance of heterochromatin requires RNAi, which guides histone modification on the two daughter strands upon DNA replication. However, the underlying mechanism is poorly understood. In *S. pombe*, the alternating arrangement of origins of replication and noncoding RNA transcribed during S phase in the heterochromatic pericentromeric region provokes the collision of RNA polymerase with replication machinery. We propose that it is resolved by cotranscriptional RNAi, allowing replication to complete and couple the spreading of heterochromatin with fork progression. In the absence of RNAi, stalled forks are repaired by homologous recombination without histone modification. The molecular basis of this model and its genome-wide impact is being further investigated and may explain the participation of RNAi and DNA replication in *S. pombe* and many other systems of heterochromatin inheritance.

Transposon Small RNA Expression in the Embryonic and Trophectoderm Lineage

A. Schorn [in collaboration with W. Reik, Babraham Institute, Cambridge, United Kingdom; Scott Lowe, Memorial Sloan-Kettering Cancer Center, New York]

Epigenetic reprogramming is essential to regain pluripotency during reproduction, but it also results in transient release of transposable elements (TE) from heterochromatin repression. In plants, transposon release in neighboring tissues triggers the production of mobile small RNA in the generative sperm cells. We wondered whether, 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 (ES) and trophectoderm stem (TS) cells and found that TEs, which produce small RNAs in ES or TS cells, are younger than the average transposon age genome-wide. Endogenous retroviruses (ERV) were particularly overexpressed in TS cells. Overexpression seems not simply due to loss of methylation in TS cells because Dnmt1$^{-/-}$ ES cells do not exhibit ERV-derived small RNAs. 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 conceptus from active transposition.

PUBLICATIONS

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Rama Umamaheswari and Rowan Herridge

PLANT DEVELOPMENTAL GENETICS

M. Timmermans A. Benkovics, M. Dotto, C. Fernandez-Marco, 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 (miRNA) 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 short 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 upper-most 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. We are currently studying the role of this specific small RNA pathway and the properties of mobile small RNAs in general in maize, *Arabidopsis,* and the moss *Physcomitrella patens*.

Small RNA Gradients Create Stable Developmental Boundaries

A. Benkovics, D. Skopelitis, C. Fernandez-Marco

Mathematical modeling of the tasiR-ARF–ARF3 interaction predicts that small RNA gradients resulting from mobility are uniquely suited to generate sharply defined boundaries of target gene expression. To test this, we are taking advantage of the *pARF3:ARF3-GUS* reporter generated previously. In an *rdr6* mutant background, which blocks tasiR-ARF production, this reporter is expressed throughout the developing leaf. In this background, we express an artificial miRNA targeting *ARF3* (miR-ARF3) from different leaf-specific promoters and in an inducible manner to modulate the position, direction, and steepness of this small RNA gradient. Target gene expression is being monitored by GUS histochemistry, and miR-ARF3 expression patterns will be determined by in situ hybridizations. The first observations suggest that small RNA gradients indeed generate sharply defined domains of target gene expression and provide evidence for dose-dependence in the miRNA gradient–target gene interaction.

Patterning via small RNA gradients is also being addressed using a reporter system that monitors the readout of a miR166 gradient. An miR166-insensitive HD-ZIPIII reporter (PHB*-YFP) that is active throughout the leaf induces an adaxialized leaf phenotype. In this background, we express a modified version of miR166 (miR166*) that specifically cleaves the PHB*-YFP transcripts. Given the importance of miRNA dosage to gene silencing, we are expressing miR166* in an inducible manner in the adaxial or abaxial epidermis. This allows us to quantitatively measure the relationship between miRNA gradients and the spatial patterning of their targets. The first

results show that miR166* expression from the abaxial epidermis is sufficient to suppress the PHB*-YFP leaf defects, indicative of movement of this small RNA. Efforts to visualize the PHB*-YFP and miR166* expression domains are ongoing.

Considering that patterning of the adaxial–abaxial axis involves two opposing small RNA gradients, these might serve to confer robustness onto the leaf development program by stabilizing the adaxial–abaxial boundary throughout organogenesis and under a range of environmental conditions. Our preliminary results suggest that the adaxial–abaxial boundary as visualized by an abaxial reporter is less sharp in *sgs3* mutants, which lack the tasiR-ARF gradient, than in wild-type plants. We are also comparing variability in leaf parameters under normal and mild stress conditions between wild-type and *sgs3* mutant plants. The outcome of these experiments will reveal whether small RNA gradients provide robustness to the leaf developmental program under a range of environmental conditions.

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. To investigate miRNA mobility in multiple distinct developmental contexts, we are expressing an artificial miRNA targeting a cell-autonomous green fluorescent protein (GFP) reporter (miR-GFP) from a number of promoters with distinct spatiotemporal patterns of expression. Expression of miR-GFP from the epidermis-specific *ATML1* promoter, mesophyll-specific *RBCS* promoter, or vasculature companion cell-specific *SUC2* promoter showed that miRNAs move bidirectionally between all three cell layers and that the number of cells across which a small RNA moves is determined in part by its abundance. Moreover, analysis of GFP fluorescence in the vasculature supports the idea that miRNA-mediated gene regulation is a dose-dependent process and depends on the relative abundance of the miRNA versus the target mRNA. No GFP silencing was observed in the symplastically isolated stomata, suggesting that miRNA movement occurs via plasmodesmata, small channels that connect most plant cells.

Interestingly, production of miR-GFP in young leaf primordia surrounding the shoot apical meristem (SAM) resulted in silencing of GFP inside the SAM, demonstrating that miRNAs traffic from determinate organs into the shoot stem-cell niche. In addition, miR-GFP was able to move from the vasculature below the SAM into the niche. These findings suggest that miRNAs may function as mobile signals between differentiated tissues and stem cells, possibly integrating environmental/physiological cues and plant development. Analyses of miRNA mobility in other developmental contexts are still ongoing, but the data obtained thus far suggests that small RNA mobility is developmentally regulated and follows rules that are distinct from those that govern protein trafficking. 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.

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. This project aims to identify additional signals in adaxial–abaxial patterning. The adaxial promoting HD-ZIPIII transcription factors contain a predicted START lipid-binding domain (Fig. 1). 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. Analysis of *Arabidopsis* lines that express PHB-YFP (yellow fluorescent protein)

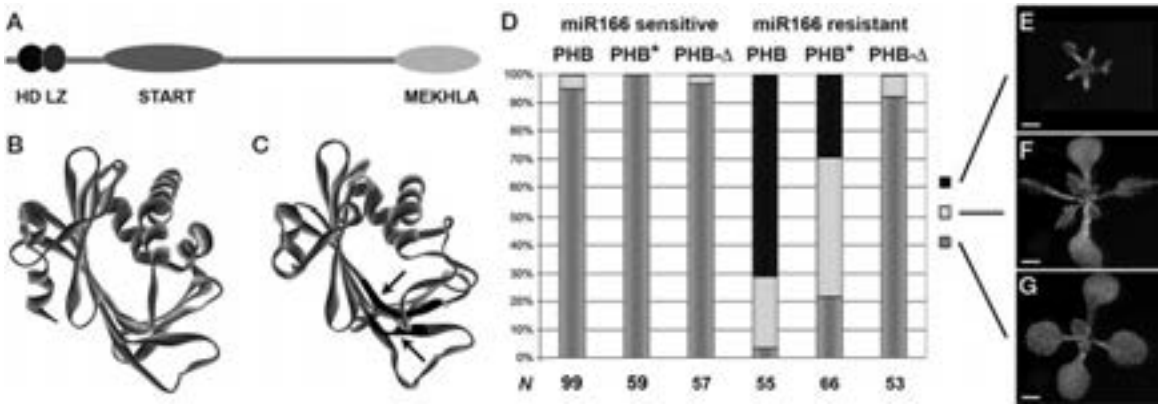

Figure 1. Activity of the HD-ZIPIII transcription factors is regulated by the START lipid-binding domain. (*A*) Schematic representation of the domain structure of HD-ZIPIII proteins. (HD) Homeodomain; (LZ) leucine zipper; (START) START lipid-binding domain; (MEHKLA) MEKHLA/PAS transcription regulatory domain. (*B*) Representation of the structure of the human PC-TP START domain modeled in SwissExpasy. (*C*) Predicted fold of the PHB START domain modeled in SwissExpasy. Amino acids mutated in the PHB-YFP derivative are highlighted in black arrows. (*D*) Distribution of phenotypes observed in plants transformed with various PHB derivatives that either remain under control of miR166 (*left*) or carry mutations that render the transcripts resistant to miR166 (*right*). (PHB) Full-length PHB protein; (PHB*) PHB derivative with critical amino acids in the START ligand-binding pocket mutated; (PHB-Δ) PHB derivative with the START domain deleted; (*N*) number of transformants analyzed. (*E*–*G*) Phenotypes in the transformants were grouped into three categories: (*E*) Severely adaxialized, indicated in black; (*F*) weakly adaxialized, indicated in light grey; (*G*) largely normal, indicated in dark grey. Mutation or deletion of the START domain suppresses the ability of miR166-resistant PHB to adaxialize developing leaves.

fusion proteins with mutations in key residues lining the START ligand-binding pocket indicates that the START domain is required for proper HD-ZIPIII function. Such mutations block PHB-mediated target gene expression, indicating positive regulation of HD-ZIPIII activity by an unknown ligand.

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. Likewise, ligand binding is not essential for PHB dimerization. However, ligand binding does affect PHB's ability to bind DNA and possibly the interaction with protein partners. As part of these analyses, we developed a yeast-based assay and conducted a high-throughput screen for chemical compounds that bind the HD-ZIPIII START domain and modulate protein activity. Using yeast growth as readout, we identified compounds that reduced yeast growth (antagonists) and others that promoted growth (agonists). Validation experiments in yeast and plants are under way to eliminate false positives and demonstrate that these chemicals act via modulation of HD-ZIPIII activity. In a parallel approach, we have generated plants expressing a biotin-tagged form of the START domain and will be performing immunoprecipitations and mass spectrometry to identify the endogenous ligand bound by HD-ZIPIII proteins. These latter experiments are under way in collaboration with the CSHL proteomics facility.

tasiRNA Pathways in Maize and New Players in Leaf Polarity

M. Dotto [in collaboration with M. Hammell, Cold Spring Harbor Laboratory; M. Aukerman, M. Beatty, and R. Meeley, DuPont-Pioneer]

Small RNAs control the activities of a wide variety of genetic elements, including protein-coding genes, transposons, and viruses. These diverse functions are achieved by distinct classes of small RNAs generated through discrete genetic pathways. tasiRNA biogenesis is triggered when *TAS* transcripts are targeted by either a single 22-nucleotide miRNA (one-hit model) or two 21-nucleotide miRNAs (two-hit model). These miRNA-guided cleavage events trigger conversion of the tasiRNA precursor transcript into long

double-stranded RNAs (dsRNAs) by RDR6 and SGS3/LBL1 and set the register for the subsequent production of phased 21-nucleotide tasiRNAs by DCL4. Only the *TAS3* pathway is conserved across land plant evolution. The binding of two miR390-loaded AGO7 complexes triggers the biogenesis of *TAS3*-derived tasiRNAs, a subset of which are the tasiARFs. We previously identified four *TAS3* loci in the maize genome; however, additional *TAS* loci described in other species are not conserved in maize. We used a deep-sequencing and bioinformatics approach to perform a genome-wide search for clusters of phased small RNA in combination with a comparison of the small RNA content between wild-type and *lbl1* mutant apices. This identified five novel *TAS* loci, all belonging to the *TAS3* family. No *TAS* loci triggered by 22-nucleotide miRNAs were identified. In-depth target analyses indicate that the tasiARFs are the only functional tasiRNAs in the maize vegetative apex, where they regulate expression of *ARF3* homologs. This study also revealed unexpected contributions for LBL1 in the regulation of miRNAs and 24-nucleotide transposon- and retrotransposon-derived small interfering RNAs (siRNAs), illustrating substantial cross-talk between small RNA pathways. The mechanisms underlying these regulatory networks remain to be elucidated.

In a separate approach, we are characterizing small RNAs associated with diverse AGO proteins. Using a specific peptide antibody, we analyzed the small RNAs associated with ZmAGO10 by deep sequencing the small RNAs present in immunoprecipitates. We found that the immunoprecipitation fraction was enriched for just a subset of small RNAs: miR394, miR166, and tasiARFs. We had previously shown the function of the latter two in the specification of maize leaf polarity. However, miR394 remains poorly characterized thus far. In situ hybridization showed that this miRNA also accumulates in a polar pattern, establishing a gradient on the adaxial side of developing leaves. This presents the possibility that organ polarity is regulated also by a fourth small RNA signal. We are currently characterizing the maize single and double mutants in components of this pathway: *mir394a* and *mir394b*, the two F-BOX proteins predicted as targets, and both *ZmAGO10* genes. It was reported that AGO10 in *Arabidopsis* associates exclusively with miR166. We are currently analyzing how structural differences between these miRNA precursors and the AGO10 proteins in both species account for the differential AGO10/miR394 binding in maize.

Effects of Natural Variation Present in Maize Inbreds on the tasiRNA Pathway

K. Petsch

Mutants that disrupt tasiRNA biogenesis exhibit defects in adaxial–abaxial patterning. Interestingly, 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 W22, Mo17, and A619. To understand how inbred background influences expressivity of tasiRNA pathway mutants, we are using the severe *ragged1* allele of *leafbladeless1 (lbl1-rgd1)*. In the B73 inbred background, *lbl1-rgd1* conditions a classical leaf polarity phenotype with most leaves developing as radial, abaxialized organs. In W22, however, *lbl1-rgd1* embryos lack a shoot meristem and germinate with just a root. We have generated an F_2 population to map loci that contribute to the variable expressivity of *lbl1-rgd1* in the two inbreds. This identified a major modifier locus on chromosome 8, which we are fine-mapping in collaboration with DuPont. In addition, we are generating NIL populations to further define the effect of this locus on the *lbl1-rgd1* phenotype and to identify the causative gene.

In a complementary approach, we performed a transcriptome analysis of *lbl1-rgd1* embryos and nonmutant siblings in B73 and W22. This identified 214 genes that are differentially expressed in *lbl1-rgd1* 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. Most notably, ~30% of genes that are down-regulated in W22 mutant embryos have "meristem-associated" functions. Further investigation into specific genes is ongoing. Importantly, this study identified 40 genes within the chromosome-8 modifier region that are expressed in the developing embryo and show differential expression between the inbreds or between *lbl1-rgd1* and nonmutant siblings. By combining these approaches, we hope to gain a better understanding of the factors

contributing to natural variation in tasiRNA pathway function.

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, because many of these have been shown to be conserved in the moss *P. 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. The genes involved in biogenesis of tasiRNAs as well as 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. For this, we are taking advantage of the unique ability of this moss to be transformed by homologous recombination and characterizing knockouts of genes involved in tasiRNA biogenesis, as well as the targets of the moss tasiR-ARFs. Our results indicate a role for tasiRNA regulation in plant architecture during the filamentous stage of growth. *sgs3* loss-of-function mutants display increased branching and decreased formation of caulonema, long runners that allow the moss plants to rapidly spread. These phenotypes are consistent with a defective response to the plant hormone auxin, which is evolutionarily more ancient than the land plant lineage itself. Further experiments demonstrated that tasiRNAs are important for setting a threshold for auxin response and that deregulation of their targets results in a perturbed response to nitrogen deprivation.

Much like filamentous runners in moss, formation of lateral roots in dicots is regulated by auxin and modulated by nitrogen levels in the substrate; in addition, a link between lateral root outgrowth and tasiRNA biogenesis has recently been demonstrated. It is exciting to hypothesize that tasiRNAs and their targets may be part of an ancient mechanism for regulating plant architecture in response to auxin signaling and environmental inputs such as soil nutrient content. To address this experimentally, we are now exploring lateral root formation in *Arabidopsis* mutants with perturbed tasiRNA biogenesis.

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]

Plants have the distinctive ability to form new organs throughout their lifetime, which can span hundreds or even thousands of years. The growing tip of a plant contains a population of stem cells that are located within a specialized niche, termed the shoot apical meristem (SAM). These stem cells divide to maintain the SAM and to generate daughter cells from which lateral organs, such as leaves and flowers, arise. The gene regulatory networks controlling meristem indeterminacy and organogenesis remain largely obscure. To gain insight into such networks, we have generated a high-resolution gene expression atlas for the maize shoot apex. Functional domains of the meristem were isolated by laser microdissection and analyzed by RNA deep sequencing. These include the whole meristem, the stem cell containing SAM tip, the newly initiating leaf (P0), the epidermal layer of the SAM (L1), and the subepidermal region (L2). 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. We identified 502 genes that show strong differential expression between the L1 and the L2. Using CAST clustering, we further identified genes that specifically mark the meristem, the P0, or developing leaf primordia. Most notably, this analysis identified 177 genes that specifically mark the stem cell domain of the meristem, and several genes that mark the presumptive organizing center. 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. Reverse genetic resources available for maize are being used to directly test the function of select genes in these processes. This has identified several new mutations affecting meristem maintenance and leaf morphology.

Establishment of Determinacy during Lateral Organ Development

M. Lodha

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* expression needs to be maintained in a stable "off" state throughout lateral organ development. We had previously shown that this process is mediated by the transcription factors AS1 and AS2, which form heterodimers that bind specific sites in the *KNOX* promoters. We have now uncovered the mechanism through which AS1-AS2 maintain the stable repression of *KNOX* genes during leaf development.

We found that *KNOX* gene silencing also requires the activities of the Polycomb repressive complexes, PRC2 and PRC1. PRC2 has histone H3K27 trimethylation (H3K27me3) activity, and we have shown that this repressive chromatin mark is enriched at the *BP* and *KNAT2* loci in leaves of *Arabidopsis*. Mutations in the PRC2 component CURLY LEAF (CLF) led to reduced levels of this repressive H3K27me3 mark at the *KNOX* loci and to ectopic *KNOX* expression in developing leaves. 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 is present at the silenced *KNOX* loci and that mutations in *LHP1* lead to *KNOX* misexpression in developing leaves. Importantly, levels of H3K27me3 and LHP1 at the *KNOX* loci are dramatically reduced in *as1* and *as2* mutants, identifying these DNA-binding proteins as upstream components in the *KNOX* silencing pathway. We were further able to show that AS1 and AS2 interact physically with PRC2 and that the AS1-AS2 complex mediates the recruitment of PRC2 to the *KNOX* promoters.

Genome-wide H3K27me3 profiling studies predict that as many as 20% of *Arabidopsis* genes are marked with H3K27me3 and regulated by PRC2. However, how PRC2, which does not bind DNA specifically, recognizes defined targets remained a major outstanding question in the field. This work showed that PRC2 is recruited to select targets by the sequence-specific DNA-binding factors AS1 and AS2. This recruitment mechanism resembles the Polycomb response element (PRE)-based recruitment of PRC2 originally defined in flies and provides the first such example in plants. Moreover, these data provide a framework for the cellular memory system underlying the somatically heritable repression of stem-cell-promoting homeobox genes that is required for cellular differentiation in leaves.

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GENOMICS

Thomas Gingeras and colleagues study where and how functional information is stored in genomes. These efforts help explain the biological and clinical effects of disease-causing gene mutations in humans and other organisms. Gingeras is a leader of the ENCODE (ENCyclopedia of DNA Elements) and 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 pre- and posttranscriptional and translational processes, as scaffolds upon which large protein complexes are assembled and as extracellular signals. The initial studies that led to these observations have been extended to cover the entire human genome.

There has been a growing appreciation in recent years that gene function is frequently context-dependent, with a large part of that context provided by the activities of other genes. But trying to understand how genes interact to produce function is a hugely complicated problem and one that appears likely to become more so as genomic information becomes more detailed. **Jesse Gillis** and colleagues are computational biologists who are presently challenging an oft-taken approach to the problem in which the functions of genes are interpreted in the context of networks derived from gene association data. Such networks consist of millions of interactions across thousands of genes, derived from protein-binding assays, RNA coexpression analysis, and other sources. Historically, many attempts to understand gene function through networks have leveraged a biological principle known as "guilt by association." It suggests that genes with related functions tend to share properties (e.g., physical interactions). In the past decade, this approach has been scaled up for application to large gene networks, becoming a favored way to grapple with the complex interdependencies of gene functions in the face of floods of genomics and proteomics data. Gillis' work centers on identifying the limits of the approach and making fundamental improvements to its operation, as well as applying those improvements to neuropsychiatric gene network data.

Gholson Lyon's lab focuses on analyzing human genetic variation and its role in severe neuropsychiatric disorders and rare diseases, including Tourette syndrome, attention-deficit hyperactivity disorder (ADHD), obsessive compulsive disorder (OCD), intellectual disability, autism, and schizophrenia. By recruiting large groups of related individuals living in the same geographic location (e.g., Utah), Lyon's lab can study the breadth and depth of genetic variants in a similar environmental background. Using the exome—the parts of the genome that code for protein—and whole-genome sequencing, his lab looks for mutations that segregate with syndromes in the various populations. A second focus of the Lyon lab is to study the mechanistic basis of a new rare disease that they described in 2011. This is the first human disease involving a defect in the amino-terminal acetylation of proteins, a common modification of eukaryotic proteins carried out by amino-terminal acetyltransferases (NATs). The team has been using several different cellular model systems to better understand the disease pathophysiology and the basic process of amino-terminal acetylation. This year, Lyon collaborated with a team of researchers from other universities and companies to use precision medicine to successfully treat a patient with severe OCD. His symptoms were treated with deep brain stimulation, and the team used whole-genome sequencing to try to understand the molecular basis of his disease. The patient experienced significant relief from his symptoms and his quality of life returned, suggesting that similar methods may hold tremendous promise in the future.

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 5 years ago. They have brought online a new generation of Illumina sequencers and optimized their function to a level at which eight to 10 trillion DNA bases can be sequenced in a month. McCombie's team has been involved in international efforts culminating in genome sequences for maize, rice, and this year for bread wheat—three of the world's most important food crops. They have also had an important role in projects to sequence the flowering plant *Arabidopsis thaliana*, 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 resequence genes in patient samples that are of special interest to human health, including *DISC1* (a strong candidate gene for schizophrenia), looking for genetic variants implicated in bipolar illness and major recurrent depression. With Memorial Sloan-Kettering Cancer Center, they are using a method called hybrid selection, 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, they seek answers to the following questions: How are genes conserved and lost over time? What are the fates of duplicated genes? What is the impact of structural variation on phenotypic variation? Ware's team also studies gene regulation in plants, focusing on gene regulatory networks, targeting transcription factors and microRNA genes with the objective of understanding how these parts of the plant genome work together in determining spatial and temporal expression of genes. The lab had an important role in the project to produce a haplotype map reference genome of maize, spearheading the most comprehensive analysis of the crop yet. This has provided important information on the variation of the reference genome, as well as comparative data showing changes in the genome acquired through domestication and breeding. They have devoted special attention to examining diversity within maize, grape, and tomato, aiming to accelerate the development of strategies to introduce new germplasm that is needed to meet demands of increasing population and a changing environment. The lab also has brought fully sequenced genomes into an integrated data framework, to enhance the power of their comparative studies. This past year, Ware was named as its principal investigator for the National Science Foundation-funded Gramene project, a comparative genomics resource for agriculturally important crops and models to support sustainable food and fuel production. Ware, as principal investigator for plants, has also 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 AND REGULATION AND FUNCTIONAL ROLES OF NONCODING RNAs

T.R. Gingeras P. Batut, S. Chakrabortty, C. Davis, A. Dobin, J. Drenkow, G. Nechooshtan, A. Prakash, A. Scavelli, F. Schlesinger, L.-H. See, H. Xue, C. Zaleski

Human ENCODE Project

P. Batut, C. Davis, A. Dobin, J. Drenkow, A. Scavelli, L.-H. See, C. Xue, C. Zaleski

Our laboratory is currently involved in phase 3 of the Encyclopedia of DNA Elements (ENCODE3) project that began in July of 2012. This project endeavors to identify and characterize the functional elements present in the human genome sequence. Our contribution to this effort has been and continues to be the cataloging of the sites of long (>200 nucleotides) and short (<200 nucleotides) RNA transcription, the positions of 5′ and 3′ termini, as well as the splice sites present in human cells/tissues. In previous phases of the ENCODE project, the biological materials that served as a source for analyses were mostly cancer cell lines, whereas in this phase, the project has sought to analyze both human primary cell lines and tissues. This year, long RNAs were sequenced (RNA-Seq) from more than 30 primary cells, and a similar collection of human tissues was analyzed. A significant proportion of the year has been spent in attempting to centralize the analyses of the RNA-Seq data sets to the Data Coordination Center (DCC) established by the ENCODE project at Stanford University. To accomplish this, an evaluation of the available software tools that are needed in an analysis pipeline to map, quantify, and model data obtained from the RNA-Seq is currently being carried out by the DCC. Related to this evaluation effort was our participation this year in two independent evaluations carried out by the RGASP (RNA-Seq Genome Annotation Assessment Project) consortium of various RNA-Seq data-mapping software as well as transcript modeling/quantification tools. To assess the performance of current mapping software, we were invited as a developer of the STAR RNA-Seq mapping program to process four large human and mouse RNA-Seq data sets as part of the RGASP evaluation. A total of 26 mapping protocols based on 11 programs and pipelines were evaluated (Engström et al. 2013). STAR (spliced transcripts' alignment to a reference; created in our laboratory by Dobin et al. 2013) was determined to be one of the best performing mapping tools tested.

Model Genome (mod)ENCODE: Human-Fly-Worm Comparative Analyses of Transcriptomes

C. Davis, A. Dobin, C. Zaleski

Both the ENCODE and modENCODE consortia have generated a community resource containing large amounts of RNA-Seq data from a wide variety of samples, resulting in a comprehensive annotation for the human, worm, and fly genomes. Extensive data integration reveals fundamental principles of transcription, conserved across highly divergent animals. In particular, by clustering expression profiles, we discovered conserved coexpression modules shared between the organisms, many of which are enriched in developmental genes. We used these to align the stages in worm and fly development, finding the expected embryo-to-embryo and larvae-to-larvae pairings in addition to a novel pairing between worm embryo and fly pupae. Furthermore, we found that the extent of per-base-pair noncanonical noncoding transcription is similar between the organisms. Finally, we found that the gene expression levels in the organisms, both coding and noncoding, can be consistently predicted by their upstream histone marks using a common "universal model."

Mouse-ENCODE Project: Human–Mouse Comparative Analyses of Transcriptomes

C. Davis, A. Dobin, C. Xue, C. Zaleski

Mammalian genomes are pervasively transcribed, with much of the transcribed regions appearing to

lack the associated signatures of selective constraint often considered indicative of biological function. However, by analyzing the transcriptomes of a large and diverse panel of human cell lines and mouse tissues, we have observed that many of the transcriptional features associated with pervasive transcription, specifically intergenic, antisense, and chimeric transcription, are conserved between human and mouse even in genomic regions that lack signatures of evolutionary constraint. We also observed that the degree of conservation of the levels of expression and splicing is independent from the conservation of promoter and splicing sequences. These results reveal an evolutionary tendency to maintain transcriptional levels and organization that are not easily traceable to the conservation of primary sequence.

Database and Software Development

A. Dobin, C. Xue, C. Zaleski

Database Development

The infrastructure of our laboratory's analysis programs is used for the continued development and maintenance of the large databases collected as part of the human–mouse and model genome (mod)ENCODE projects. The notable achievements in this area include the following:

- Continued management and organization of sequencing data consisting of fastq files (raw data) and genome-mapped data files (bam, bigwig, contig). During this year, we have added ~30 TB (terabytes) of sequencing data.
- Continued improvement and development of the RNA sequencing production pipeline. This consists of sequence mapping into multiple formats, generation of "contigs" and "splice junctions," several new multiple QC steps, and file transfer of data files to outside locations (DCC and our Barcelona collaborators).
- Implementation of an ENCODE data submission client to interface with the primary repository at the University of California, Santa Cruz. A set of web-based forms connects to a server at CSHL, which translates the information to schema-compliant structure. The data are then submitted to a REST (representational state transfer) service and are viewable via the DCC's public interface.
- Integrated our lab experimental processes with a new CSHL sequencing LIMS (laboratory information management system). Data tracking, processing of unique IDs, and management of completed sequencing runs were all migrated to the new system.
- Continued coordination with the CSHL IT department to manage our primary storage hardware migration. More than 40 TB of data will be migrated to a new hardware system and connected to the shared computing cluster as well as our own lab's servers. Code migration, testing, and QC are ongoing processes.

Software Development

- STAR development and user support: STAR is the RNA-Seq aligner developed and published in 2013 by Dobin et al. This paper has been cited 40 times (Google Scholar) in the less than 1 year and the software has been downloaded more than 3000 times in this period. Efforts were concentrated on improving the algorithm, introducing new features, and fixing bugs, with 26 patches, and the paper was publicly released. Extensive user support is provided by e-mail and through the STAR user group.
- Circular RNA (circRNA): Interest in circRNAs has been re-ignited by recent publications demonstrating that circRNAs are present in various species and cell types, as well as their possible role as microRNA sponges. A. Dobin, a member of our lab, has developed an accurate and efficient method of circRNA discovery in high-throughput RNA-Seq data, based on the chimeric detection algorithm of our RNA-Seq aligner STAR. The method does not rely on annotations or intronic motifs and thus allows for unbiased "de novo" detection of circRNAs. Using this algorithm, we analyzed the circRNA repertoire in hundreds of human cell lines of the ENCODE transcriptome data set. Thousands of circRNAs are reproducibly detected in each of the samples. We demonstrate that circRNAs are significantly enriched in non-polyadenylated RNA populations compared to the polyadenylated RNAs, corroborating the circular nature of the molecules. Most of the detected circRNAs possess canonical intron motifs and connect protein-coding exons, implicating the standard splicing machinery in the biogenesis of the

circRNAs. Some but not all of the circular molecules appear to correspond to alternatively spliced exons and can be attributed to the "lariat model." By comparing the circRNA and linear RNA abundances across a variety of cell types, we show that many circular RNAs are strongly regulated.

De Novo DNA Demethylation and Noncoding Transcription Define Active Intergenic Regulatory Elements

F. Schlesinger

Deep sequencing of mammalian DNA methylomes has uncovered a previously unpredicted number of discrete hypomethylated regions in intergenic space (iHMRs). In collaboration with the Hannon laboratory at CSHL, we combined whole-genome bisulfite sequencing data with extensive gene expression and chromatin-state data to define functional classes of iHMRs and to reconstruct the dynamics of their establishment in a developmental setting. Comparing HMR profiles in embryonic stem and primary blood cells, we show that iHMRs mark an exclusive subset of active DNase-hypersensitive sites (DHS) and that both developmentally constitutive and cell-type-specific iHMRs display chromatin states typical of distinct regulatory elements. We also observe that iHMR changes are more predictive of nearby gene activity than the promoter HMR itself and that expression of noncoding RNAs within the iHMR accompanies full activation and complete demethylation of mature B-cell enhancers. Conserved sequence features corresponding to iHMR transcript start sites, including a discernible TATA motif, suggest a conserved, functional role for transcription in these regions. Similarly, we explored both primate-specific and human population variation at iHMRs, finding that although enhancer iHMRs are more variable in sequence and methylation status than any other functional class, conservation of the TATA box is highly predictive of iHMR maintenance, reflecting the impact of sequence plasticity and transcriptional signals on iHMR establishment. Overall, our analysis allowed us to construct a three-step timeline in which (1) intergenic DHS are preestablished in the stem cell, (2) partial demethylation of blood-specific intergenic DHS occurs in blood progenitors, and (3) complete iHMR formation and transcription coincide with enhancer activation in lymphoid-specified cells (Schlesinger et al. 2013).

High-Fidelity Promoter Profiling Reveals Widespread Alternative Promoter Usage and Transposon-Driven Developmental Gene Expression

P. Batut, A. Dobin

Many eukaryotic genes possess multiple alternative promoters with distinct expression specificities. Therefore, comprehensively annotating promoters and deciphering their individual regulatory dynamics are critical for gene expression profiling applications and for our understanding of regulatory complexity. We introduce RAMPAGE (RNA annotation and mapping of promoters for the analysis of gene expression), a novel promoter activity profiling approach that combines extremely specific 5′-complete cDNA sequencing with an integrated data analysis workflow, to address the limitations of current techniques. RAMPAGE features a streamlined protocol for fast and easy generation of highly multiplexed sequencing libraries, offers very high transcription start site specificity, generates accurate and reproducible promoter expression measurements, and yields extensive transcript connectivity information through paired-end cDNA sequencing. We used RAMPAGE in a genome-wide study of promoter activity throughout 36 stages of the life cycle of *Drosophila melanogaster* and describe here a comprehensive data set that represents the first available developmental time course of promoter usage. We found that >40% of developmentally expressed genes have at least two promoters and that alternative promoters generally implement distinct regulatory programs. Transposable elements, long proposed to have a central role in the evolution of their host genomes through their ability to regulate gene expression, contribute at least 1300 promoters shaping the developmental transcriptome of *D. melanogaster*. Hundreds of these promoters drive the expression of annotated genes, and transposons often impart their own expression specificity upon the genes they regulate. These observations provide support for the theory that transposons may drive regulatory innovation through the distribution of stereotyped *cis*-regulatory modules throughout their host genomes (Batut et al. 2013).

RNA-Mediated Intercellular Signaling and the Use of Extracellular Vesicle RNAs as Possible Cancer Biomarkers

S. Chakrabortty, A. Dobin, A. Prakash

Currently, two distinct populations of extracellular vesicles (EVs)—exosomes (<100 nm) and microvesicles (100–1000 nm) have been classified. These two types of EVs are believed to possess key differences in their mode of release from host cells. However, the qualitative and quantitative differences in the cargo RNAs between these two subpopulations of EVs have been relatively unexplored.

EV RNAs as Possible Cancer Biomarkers

The repertoire of EVs released by four cancer cell lines (K562, HELA, MCF7, and U2OS) and two primary cell lines (BJ and HUVEC) were studied. These primary cell lines are frequently the cell types most likely to be present in the tumor microenvironment. Using previously published protocols, we isolated separately the two families of EVs. Using nanoparticle tracking analysis, we quantified and analyzed the size distribution of the two families; we found that the exosomes had a size distribution ranging from 10 nm to 300 nm, with a median of 135 nm, and were released at an average across cell lines from 10 to 100 exosomes per cell. We found that microvesicles had a size distribution ranging from 50 nm to 400 nm with a median of 225 nm, but that they were less numerous than exosomes. A very large degree of overlap in size between these two subgroups, as well as all present techniques being unable to clearly separate out the two populations solely defined by their difference in size, led us to ask the question of whether the two subgroups were just an artificial construct or whether they were really two physiologically and biochemically distinct vesicles being released by cells. To answer this question, we used RNA-Seq to investigate the transcriptomes of both the EV subgroups. Unexpectedly, there was a very low correlation between the transcript types composing the contents of the two EV subgroups. This result is consistent with the likelihood that there are two distinct populations of EVs that are not merely different in their size distribution but are also distinct in their RNA content. A comparison of the RNA transcripts of exosomes to source cells in both cancer and primary-cell-derived exosomes showed that there were mainly two families of RNAs that were enriched in the exosomes when compared to source cell, microRNA (miRNA), and Y-RNA. Of the Y-RNA family, RNY5 was found to be the predominant molecule. Other interesting molecules that were seen to be enriched in exosomes were miRNA-17-92a, miRNA-93, and miRNA-103b. Comparison of exosomal and microvesicle RNA contents derived from different cell types revealed that the contents were cell-type specific, increasing the likelihood of identifying specific disease cell-derived exosomes within human body fluids.

Intercellular Signaling

The intercellular transfer of exosomes and their RNA contents have been investigated by light and electron microscopy, and their contents were studied using RNA-Seq. To demonstrate the release of exosomes in the extracellular environment, K562 cells were labeled with membrane lipid dye PKH26. Live cell imaging was performed and demonstrated the release of labeled vesicles from the cells to the extracellular environment followed by the uptake of the exosomes by different recipient cells using a transwell cell-culture system. The labeled exosomes have also been directly incubated with multiple human and mouse recipient cell types (K562, BJ, 3T3) that were labeled with lipid dye PKH67 and Hoechst 33342. In both scenarios, live imaging on the recipient cells clearly revealed the uptake of labeled exosomes by the recipient cells.

To investigate the subcellular localization of the exosomes upon uptake in the recipient cells, multiple subcellular organelles in the recipient cells were labeled before being incubated with prelabeled exosomes, and live imaging was performed. Live imaging revealed that (1) the exosomes are primarily localized in the cytoplasm in the recipient cells upon uptake and do not colocalize with any particular organelle, (2) the exosomes do not cross the nuclear membrane in the recipient cells, but occupy a characteristic perinuclear localization, and (3) the exosomes, upon uptake, are not fused with lysosomes or P bodies. To demonstrate the uptake of exosomal RNA cargo in recipient cells, metabolic labeling of RNA with ethynyl-uridine (EU) was performed. Briefly, donor K562 cells were incubated with EU for 24 h, and exosomes were isolated from the conditioned medium. The isolated exosomes were then added to recipient cells (NIH-3T3) and incubated for 2 h. The exosomal RNA was then detected using click chemistry in the recipient cells and

imaged by fluorescent microscopy. Actinomycin D was used to block the endogenous transcription from any EU carry over during the exosome preparation.

The transfer of RNA through exosomes among cells was also investigated by another orthogonal technique, namely, RNA-Seq. One member of the Y-RNA small noncoding gene *RNY5* is expressed abundantly in humans as well as in many lower evolutionary organisms, but it is genetically missing in rodents. Human *RNY5* RNA transcripts are particularly abundant in exosomes secreted by K562 cells and thus can serve as excellent genetic markers for intercellular (and interspecies) RNA transfer through exosomes. K562 exosomes were isolated and incubated with mouse HB-4 cells for various durations (0, 6, 12, 24, and 48 h). Small RNA was isolated from recipient mouse cells, and the presence of *RNY5* transcripts in mouse HB-4 cells was detected using RNA-Seq. Interestingly, the time-course experiment revealed the stability of *RNY5* in mouse cells. Maximum expression of *RNY5* was detected at a 12-h incubation period in the recipient cells.

One of the most well-known and studied functions of tumor-derived exosomes is its role in inducing apoptosis upon transfer in the recipient immune cells. The ability of tumor exosomes to induce apoptosis in immune cells is generally regarded as a mechanism of immune evasion for the tumor cells. The mechanism through which apoptosis is induced is thought to be protein mediated (Fas-Ligand pathway). In this project, we decided to investigate the role of RNA cargo of tumor exosomes upon transfer in two other important primary cells of the tumor microenvironment, namely, primary fibroblast (BJ) and endothelial (HUVEC) cells.

K562 and BJ cells were incubated with K562 exosomes for 24 h, and live-dead cell counting was performed using Hoechst 33342 (live) and Yo-pro1 (dead) dye. Surprisingly, we discovered K562 exosomes also induce apoptosis in primary fibroblast cells (BJ) but not in the tumor cells (K562) themselves. Thus, the ability to induce apoptosis by tumor exosomes is not restricted to immune cell lineage alone. To investigate whether the observed phenotype is caused by exosomal RNA or exosomal proteins/lipids, RNA was isolated from K562 exosomes and lipofected into BJ and K562 cells using Lipofectamine 2000. Consistent with previous experiment, apoptosis was detected again in BJ cells alone but not in K562 cells, thus suggesting that apoptosis is caused by exosomal RNA and not exosomal proteins/lipids. Scrambled RNA and mock lipofectamine treatment were performed as negative controls—neither of which caused apoptosis in any of the cells.

One of the most abundant transcripts present in K562 exosomes is a 32-mer 5′ fragment of a small noncoding RNA, *RNY5. RNY5* is a Polymerase-III-transcribed gene of the Y-RNA family (Y1, Y3, Y4, and Y5) and is thought to have a role in DNA replication and RNA degradation (when bound with protein Ro). To investigate whether *RNY5* transcripts in exosomal cargo are responsible for causing apoptosis, K562 and BJ cells were transfected with a synthetic *RNY5* fragment (32-mer) with Lipofectamine 2000. Consistent with previous experiments with K562 exosomes and exosomal RNA, transfection of the synthetic *RNY5* fragment alone resulted in apoptosis in BJ cells but not in K562 cells. Mock lipofectamine treatment and nonspecific scrambled sequences (Allstate negative control RNA) were used as negative controls; neither of which caused cell death in any cells.

Full-length *RNY5* has a characteristic secondary structure of two double-stranded RNA stem structures called lower and upper stem, separated by an inner loop that is conserved among all Y-RNA genes. Exosomes carry a 5′ 32-nucleotide fragment of *RNY5* only, and thus, only the 5′ side of the lower and upper stem and inner loop is present in exosomes. The double-stranded upper stem in full-length *RNY5* is considered to be the motif responsible for its role in DNA replication. Because exosomes contain only the 5′ side of the stem structures, the 5′ side of the upper stem (8-mer sequence GUUGUGGG) from nucleotides 14–21 was investigated for its role in apoptosis. K562 and BJ cells were transfected with a normal synthetic y5 fragment (32-mer, synthetic Y5 fragment with 8-mer deleted; Hy5-δ-us) and 8-mer-scrambled (Hy5-us-scram). Neither hy5-δ-us nor hy5-us-scram was able to induce cell death in either of the cells anymore, thus suggesting that the 8-mer sequence is the motif in *RNY5* responsible for causing apoptosis in primary cells.

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GENE NETWORK ANALYSIS

J. Gillis S. Ballouz W. Verleyen

Research in my lab centers on using computational methods to understand gene function. Computational biology has taken up the challenge of determining gene function mainly by attempting to interpret the activities of genes in the context of networks derived from gene association data. As data sets characterizing genes grow in size and complexity, it seems self-evident that computation can assist in inference as to gene function. Gene network analysis intended to provide insight into complex disorders is a dominant interest in the field. Gene associations (of various sorts) are believed to encode functional interaction, and this interaction is frequently shown to be able to substantially predict gene function across all functions. This approach, commonly called "guilt by association" (GBA), is embedded in everything from prioritization of de novo variants to uncovering novel molecular phenotypes or mechanisms of disease. Our research focuses on identifying limitations in the GBA approach and making fundamental improvements to its operation for the interpretation of neuropsychiatric genomics data. Postdocs Sara Ballouz and Wim Verleyen joined my lab in early 2013.

Analysis of RNA-Seq Coexpression Networks

RNA-Seq offers profound biological and technical advantages over microarray technologies, such as detecting the whole transcriptome and an improved dynamic range. However, as was once the case for microarrays, RNA-Seq's utility is hobbled by our inability to determine sound consensus standards. One major reason is that RNA-Seq data have statistical properties quite different from those of microarray data, and thus the same principles do not apply for differential expression. Important questions about data quality, such as the role of biological noise in experimental design, are not readily addressed. A broader characterization of RNA-Seq data through meta-analysis offers one avenue to investigate problems of this nature.

Although differential expression analysis is a more common means for interpreting transcriptomic data, coexpression analysis is far more routine in the context of meta-analysis, with thousands of expression profiles aggregated to generate robust signatures using repurposed data. One advantage of coexpression methods is that they already tend to require meta-analysis of disparate data sets with quite different properties, so approaches for aggregating across data are already common. We have developed a series of methods appropriate for RNA-Seq meta-analysis and have produced reference networks for general use. RNA-Seq coexpression poses novel statistical and bioinformatics challenges. We have identified major confounds and developed appropriate control experiments necessary for network construction, laying the groundwork for functional analyses into RNA-Seq coexpression; we are particularly focusing on functional inference in noncoding RNA.

Bias Tradeoffs in the Creation and Analysis of Protein–Protein Interaction Networks

Networks constructed from aggregated protein–protein interaction data are commonplace in biology. But the studies these data are derived from were conducted with their own hypotheses and foci. Focusing on data from budding yeast present in BioGRID, we determine that many of the downstream signals present in network data are significantly impacted by biases in the original data. We determine the degree to which selection bias in favor of biologically interesting bait proteins goes down with study size, and we also find that promiscuity in prey contributes more substantially in larger studies. We analyze interaction studies over time with respect to data in gene ontology and find that reproducibly observed interactions are less likely to favor multifunctional proteins. We find that strong alignment between coexpression and protein–protein interaction data occurs only for extreme coexpression values and use these data to suggest candidates for targets likely to reveal novel biology in follow-up studies.

Assessing Identity, Redundancy, and Confounds in Gene Ontology Annotations Over Time

Gene ontology (GO) is a key means by which systems biologists operationalize gene function, making it a heavily relied upon tool in innumerable analyses and data interpretation exercises. GO annotations are often used as a gold standard, but it has widely appreciated imperfections. Ironically, it is very difficult to assess the properties of GO itself because there is no other comprehensive gold standard against which to hold it. Broadly speaking, assessment of GO has focused on three distinct attributes: the accuracy of annotations assigned to GO, GO's structure independent of annotation, and the utility of GO and its annotations for the interpretation of data. Despite misgivings about the incompleteness of GO annotations, the use of GO "sets" as representing "functions" is now endemic. This is put to use in numerous applications such as "gene group enrichment," gene network analysis, and gene function prediction. It is essential to understand the extent to which such applications are valid.

The statement "the differentially expressed genes were enriched for genes with functions in cell growth" does not necessarily mean the same thing today as it did 5 years ago, because the definition of "cell growth genes" has changed in GO. Valid experimental results often become obsolete over time, but the reported facts of the experiment should not. But that is what happens when the gene ontology changes. This is of course to be expected, and the problem can be ameliorated by reporting which version of the gene annotations was used. We find that genes can alter their "functional identity" over time, with 20% of genes not matching to themselves (by semantic similarity) after 2 years. We consider ensuring independence of GO from the data sets to which it is being applied as an absolute minimum standard, and our results show that at least some protein interaction data do not meet this standard. We discovered that many entries in protein interaction databases are due to the same published reports that are used for GO annotations, with 66% of assessed GO groups exhibiting this confound. In our experience, among systems biologists, there seems to be a broadly appreciated disjunction between the true utility of GO and how often it is used, even if this is rarely acknowledged in the peer-reviewed literature. The use of GO annotations is often regarded as a minimally interesting validation of results, but not safe to use for discovery purposes. We believe the problems we have identified are among the underlying sources of these mixed feelings about GO. If it is "too easy" to obtain interesting results using GO, and those results do not consistently hold up, then GO's utility for such purposes is limited.

Meta-Analysis across Computational Approaches Predicting Gene Function

The use of our burgeoning genomics data to characterize gene function has been one of the central research objectives of computational biology in the postgenomics era. Despite this intense focus, the abundance of public data, and methods development in machine learning and "big data" analysis, progress has been surprisingly uncertain. Attaching novel functions to genes based on prior data remains difficult, and a large number of genes still have comparatively little information attached to them.

To better understand the forces driving algorithmic and data performance, developers have focused on comparative assessment. Despite some important progress enabled by these assessments, the underlying mechanisms driving methods performance are poorly understood. Each developer may use separate data resources, different algorithms, and quite specific means for parsing the data in combination, making it quite hard to explore factors affecting performance. Field-wide progress is hampered by our inability to know why a specific algorithm worked on a particular data set for a potentially narrow task.

To overcome this lack of detail in assessment, we implemented representative samples of cutting-edge "default" machine learning algorithms and obtained performances across our set comparable to those of previous critical assessments at identical tasks with well-characterized data and fully motivated parameter choices. Because we now have in-house versions of multiple algorithms, we can explore variance and data dependencies in a way not typically possible.

We focused on data based on networks derived from protein–protein interaction, sequence similarity, aggregated coexpression, and semantic similarity to study underlying patterns of performance. Our infrastructure allows us to characterize in detail why aggregation improves performance, where results are robust

and reproducible, and what artifacts are potentially problematic in data interpretation. We plan to make this replication across methods available as a public resource, allowing geneticists using function prediction methods to better assess reproducibility and data dependencies in target genes of interest.

Assessing the First Computational Gene Function Prediction Assessment

The computational assignment of gene function remains a difficult but important task in bioinformatics. The establishment of the first critical assessment of functional annotation (CAFA) was aimed at increasing progress in the field. We demonstrated an independent assessment of the results of CAFA, aimed at identifying challenges in assessment and at understanding trends in prediction performance. We found that well-accepted methods based on sequence similarity (i.e., BLAST) have a dominant effect. Many of the most informative predictions turned out to be either recovering existing knowledge about sequence similarity or were "postdictions" already documented in the literature. These results indicate that deep challenges remain in even defining the task of function assignment, with a particular difficulty posed by the problem of defining function in a way that is not dependent on either flawed gold standards or the input data itself. In particular, we suggest that using the gene ontology (or other similar systematizations of function) as a gold standard is unlikely to be the way forward.

Schizophrenia Coexpression to Prioritize Candidate Variants

RNA coexpression data are commonly used to construct gene networks, but it is often considered to be more difficult to interpret than protein interactions. This is in part due to the lack of consensus on methods for constructing networks from expression profiles and the relatively poor performance of coexpression for function prediction, as measured by its ability to recapitulate data with GO, KEGG, or other databases. On the other hand, coexpression affords a major advantage over current large-scale protein interaction databases: It can be used to create "condition-specific" networks. In our preliminary results, we show that by appropriate consideration of data pretreatment, aggregation, and network construction, coexpression networks become a powerful tool for gene function analysis, on par with or better than protein interaction networks in terms of overall properties, while providing condition specificity. We leverage these properties to examine the role of de novo schizophrenia variants in the most comprehensive analysis to date of coexpression patterns in schizophrenia, combining seven studies of prefrontal cortex in affected individuals and unaffected controls.

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HUMAN GENETICS AND GENOMIC MEDICINE

G. Lyon M. Doerfel P. Kota
H. Fang J. O'Rawe
L. Jimenez Y. Wu

Our laboratory focuses on analyzing human genetic variation and understanding how genetic mutations contribute to severe idiopathic neuropsychiatric disorders. We do this by studying large pedigrees living in the same geographic location, where one can study the expressivity and segregation of variants in a similar environmental background and with fewer population stratification concerns. Toward this end, we collect pedigrees in Utah and elsewhere and then use exome and whole-genome sequencing to find mutations that segregate with syndromes in the pedigrees. We focus on the discovery of families with rare diseases and/or increased prevalence for syndromes such as intellectual disability, autism, and schizophrenia. Once we identify mutations that likely contribute to a disease, we undertake detailed functional studies of these mutations and the biological processes affected. Several projects are still at an early stage, but some of the projects that are sufficiently far along to discuss publicly are listed below.

Ogden Syndrome and the Amino-Terminal Acetylation of Proteins

M. Doerfel, Y. Wu [in collaboration with R. Marmorstein, Philadelphia, Pennsylvania; T. Arnesen and N. Reuter, Norway; P. van Damme, Belgium]

We have previously identified a lethal X-linked disorder of infancy comprising a distinct combination of particular craniofacial features producing an aged appearance, growth failure, hypotonia, global developmental delays, cryptorchidism, and acquired cardiac arrhythmias. The first family was identified in Ogden, Utah, with five affected boys in two generations of family members. A mutation was identified as a c.109T> C (p.Ser37Pro) variant in *NAA10*, a gene encoding the catalytic subunit of the major human amino-terminal acetyltransferase (NatA). This same mutation was identified in a second unrelated family, with three affected boys in two generations. This X-linked malformation and infantile lethality syndrome has been named Ogden syndrome, in honor of the hometown where the first family resides. This is the first human disease involving a defect in the amino-terminal acetylation of proteins, a common (yet vastly understudied) modification of eukaryotic proteins carried out by amino-terminal acetyltransferases (NATs). There is significantly impaired biochemical activity of the mutant hNaa10p, suggesting that a reduction in acetylation of some unidentified proteins by hNaa10p might lead to this disease. There is currently very limited knowledge of the functional importance of Nt acetylation at the protein level and at the organismal level.

To understand the detrimental impact of the Naa10 S37P mutation, we performed structural, molecular, and cellular investigations. The recently determined *Schizosaccharomyces pombe* NatA complex crystal structure was used as a template to generate a model of human NatA, revealing a highly conserved complex. The model allowed for comparison of Naa10 wild type and Naa10 S37P within the NatA complex and suggested a decreased flexibility for Naa10 S37P in regions involved in catalysis and at the interface with the auxiliary subunit Naa15. The hydrogen bonding network between Naa10 and Naa15 was also rearranged. In vitro enzyme kinetics of Naa10 S37P demonstrated a reduced catalytic capacity, probably due to impaired peptide substrate binding. In agreement with the structural model, Naa10 S37P displayed a reduced capacity to form a stable NatA complex. Amino-terminal acetylome analyses of patient B cells and fibroblasts provided a survey of Nt acetylation in human noncancer cells. In line with previous NatA knockdown data, patient-derived S37P B cells and fibroblasts have reduced Nt acetylation for a subset of NatA-type substrates compared to cells from healthy family members, demonstrating in vivo perturbation of Naa10 (NatA)-mediated Nt acetylation in Ogden syndrome males. THOC7, one of the affected proteins, was shown to depend on Nt acetylation

for its stability. Ogden syndrome fibroblasts further displayed abnormal cell proliferation and migration capacity. Therefore, the Ogden syndrome mutant Naa10 is impaired in NatA complex formation and catalytic capacity, and patient cells display reduced in vivo Nt acetylation and cellular phenotypes potentially linked to the defects observed in the males suffering from this disease.

Low Concordance of Multiple Variant-Calling Pipelines: Practical Implications for Exome and Genome Sequencing

J. O'Rawe [in collaboration with K. Wang, Los Angeles, California]

To facilitate the clinical implementation of genomic medicine by next-generation sequencing, it will be critically important to obtain accurate and consistent variant calls on personal genomes. Multiple software tools for variant calling are available, but it is unclear how comparable these tools are or what their relative merits in real-world scenarios might be. We sequenced 15 exomes from four families using commercial kits (Illumina HiSeq 2000 platform and Agilent SureSelect version 2 capture kit), with ~120× mean coverage. We analyzed the raw data using near-default parameters with five different alignment and variant-calling pipelines (SOAP, BWA-GATK, BWA-SNVer, GNUMAP, and BWA-SAMtools). We additionally sequenced a single whole genome using the sequencing and analysis pipeline from Complete Genomics (CG), with 95% of the exome region being covered by 20 or more reads per base. Finally, we validated 919 single-nucleotide variations (SNVs) and 841 insertions and deletions (indels), including similar fractions of GATK-only, SOAP-only, and shared calls, on the MiSeq platform by amplicon sequencing with ~5000× mean coverage. SNV concordance between five Illumina pipelines across all 15 exomes was 57.4%, whereas 0.5%–5.1% of variants were called as unique to each pipeline. Indel concordance was only 26.8% between three indel-calling pipelines, even after left-normalizing and intervalizing genomic coordinates by 20 base pairs. Of CG variants that fall within targeted regions in exome sequencing, 11% were not called by any of the Illumina-based exome analysis pipelines. On the basis of targeted amplicon sequencing on the MiSeq platform, 97.1%, 60.2%, and 99.1% of the GATK-only, SOAP-only, and shared SNVs could be validated, but only 54.0%, 44.6%, and 78.1% of the GATK-only, SOAP-only, and shared indels could be validated. Additionally, our analysis of two families (one with four individuals and the other family with seven), demonstrated additional accuracy gained in variant discovery by having access to genetic data from a multigenerational family. Our results suggest that more caution should be exercised in genomic medicine settings when analyzing individual genomes, including interpreting positive and negative findings with scrutiny, especially for indels. We advocate for renewed collection and sequencing of multigenerational families to increase the overall accuracy of whole genomes.

Advancing Precision Medicine through Clinical Grade Whole-Genome Sequencing, Return of Results, and Neuromodulation

J. O'Rawe, H. Fang [in collaboration with M. Reese, California; G. Higgins, Washington, D.C.; K. Eilbeck and R. Robison, Utah]

For widespread precision medicine to become a reality, many things must be optimized, including clinical-grade sample collection, high-quality sequencing data acquisition, digitalized phenotyping, rigorous generation of variant calls, and online sharing of medical history and genomic data with research participants and others. We report the detailed phenotypic characterization, clinical-grade whole-genome sequencing (WGS), as well as the 2-year outcome of a man with severe obsessive compulsive disorder (OCD) treated with deep brain stimulation (DBS) of the nucleus accumbens/anterior limb of the internal capsule. As part of his integrated medical care, his genome was sequenced, and variants were detected in the Illumina WGS Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory. It is increasingly apparent that mental illness results from a constellation of genetic and environmental factors. Consistent with this, WGS did not reveal any one mutation of large effect, but instead showed that he carries several alleles that have been shown to elevate risk for mental illness. This includes the p.Val66Met variant in brain-derived neurotrophic factor (BDNF), the p.Glu429Ala allele in methylenetetrahydrofolate reductase (MTHFR), and the *p.Asp7Asn* allele in choline O-acetyltransferase

(ChAT). We identified thousands of other variants in his genome, including pharmacogenetic variants, and one mutation led to the discovery that he has untreated bilateral cataracts and other visual disturbances. We archived all data in the GVFclin format and also returned many results to this person. Since implantation of the deep-brain stimulator, this man has reported steady improvement, highlighted by a decline in his Yale-Brown obsessive compulsive scale (YBOCS) score from ~38 to a score of ~25. A rechargeable Activa RC neurostimulator battery has been of major benefit in terms of facilitating a degree of stability and control over the stimulation. His psychiatric symptoms reliably become worse within hours of the battery becoming depleted, thus providing confirmatory evidence for the efficacy of DBS for OCD in this person. To our knowledge, this was the first study in the clinical neurosciences that integrates detailed neuropsychiatric phenotyping, deep-brain stimulation for OCD, and clinical-grade WGS, with management and the first return of WGS results to a person with severe mental illness.

The Characterization and Analysis of an Idiopathic Intellectual Disability Syndrome via Whole-Genome Sequencing Analysis

J. O'Rawe, Y. Wu [in collaboration with A. Rope and J. Swensen, University of Utah]

We continued this year to study a new idiopathic syndrome with intellectual disability and distinctive facial dysmorphology. The propositi are two affected male brothers, aged 10 and 12, respectively, with severe intellectual disability, autism-like behavior, attention deficit issues, and very distinctive facial features, including broad, upturned nose, sagging cheeks, downward sloping palpebral fissures, relative hypertelorism, high-arched palate, and prominent ears. Their parents are nonconsanguineous and are both healthy, and the family history does not demonstrate any other members with anything resembling this current syndrome. X-chromosome inactivation assays reveal skewing in the mother, suggesting the possibility of an X-linked disorder. High-density genotyping arrays in the mother, father, and two sons have not revealed previously known copy number variants (CNVs) that might contribute to the phenotype. Whole-genome sequencing has led to the identification of several rare variants that are currently being characterized further, including in other unaffected members of the extended family.

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DEVELOPMENT OF NEXT-GENERATION SEQUENCING TECHNOLOGY

W.R. McCombie E. Antoniou, N. Azamy, M. Bell, Y. Berstein, G. Cheang, A. DeSantis, P. Deshpande, S. Ethe-Sayers, E. Ghiban, S. Goodwin, M. Kramer, D. Lewis, S. Mavruk-Eskipehlivan, S. McCarthy, O. Mendivil Ramos, P. Mocombe, S. Muller, J. Parla, S. Teng

In 2013, we completed important phases of several projects and began new initiatives. A paper done in collaboration with colleagues at the University of Edinburgh on the role of the *Disrupted-In-Schizophrenia* (*DISC1*) gene and psychiatric disorders was published in 2013. We continued and expanded that project to study the role of *DISC1* by continuing the analysis of ~260 genes that interact with *DISC1* in more than 1500 individuals. We further expanded this project under new funding from the National Institute of Mental Health (NIMH) by beginning whole-genome sequence analysis on 39 members of the translocation family.

In collaboration with colleagues at Trinity University, Dublin, we refined our analysis of the data obtained the previous year from sequencing parent-child trios with a child afflicted by schizophrenia. The results showed a link to autism and implicated a class of genes involved in chromatin remodeling. A paper describing this work was submitted in December 2013.

In collaboration with the University of Iowa and Johns Hopkins University, we also completed the sequencing for our ongoing synaptome project. Approximately twice as many exomes as we were funded for were completed. We published a paper on a new analysis method as part of this project and will complete the analyses of these data in the coming year.

In 2013, we initiated the remaining step that we believe to be necessary for a comprehensive, overall plan to understand the genetics of psychiatric disorders by implementing high-throughput functional studies in *Caenorhabditis elegans* in collaboration with the Hammell lab at CSHL. We also moved ahead our targeting technology to better enable us to sequence small regions of the genome in very large numbers of people. Thus, we now have in place initial genetic studies on exomes or whole genomes to broadly identify possible candidate genes, as well as dual follow-up approaches of ultra-high-throughput-targeted resequencing of these candidates in much larger sample sizes and high-throughput functional tests to further characterize candidates. Although this "pipeline" needs further refinement, with it in place, we look forward to applying it to our many candidates in 2014.

In the area of cancer genetics, we began collaboration on colon cancer with colleagues at Stony Brook University. This initially focused on RNA sequencing and methylation analysis.

Finally, we made considerable progress in our plant genomics and de novo assembly research. This is being driven mostly by adapting new methods to take advantage of the increasing read lengths on the Pacific Biosciences instrument. In collaboration with the Schatz lab at CSHL, we have been working on both pure Pacific Biosciences assemblies and hybrid assemblies with Illumina data included.

Investigating the Biology of *DISC1*

S. Teng, S.E. McCarthy, M. Kramer, W.R. McCombie
[in collaboration with D. Blackwood, D. Porteous, P. Thompson, and I. Deary, University of Edinburgh; A. McRae, University of Queensland]

The *DISC1* gene has been implicated in psychiatric disorders such as major depression (UP), bipolar disorder (BD), and schizophrenia (SZ). This gene was originally identified in a Scottish family with a high burden of mental disorders (St Clair et al., *Lancet 336:* 12–16 [1990]). We have undertaken several projects to study the genetics of *DISC1*. We previously described targeted sequencing of the 528-kb *DISC1* gene region in more than 1500 individuals (221 BP, 240 SZ, 192 UP, and 889 controls). We found a nominal association of noncoding single-nucleotide polymorphisms (SNPs) (rs16856199) with major depression, as well as several extremely rare variants that seem to be present only (or almost only) in affected patients. We believe that the *DISC1* locus represents a microcosm of the complexity that has made a better understanding of the genetics of

major psychiatric disorders so difficult to unravel. There are multiple types of variants at this one locus. Some of these are common variants that provide a slight increase in risk for a disorder (depression, in this case). Other variants in the same gene are extraordinarily rare in the population, but they appear to confer an extremely high risk for a disorder, probably approaching 100%. In addition, there are probably interactions among multiple variants in the *DISC1* gene and among variants in *DISC1* and other genes. This genetic complexity is then amplified across hundreds or even thousands of genes contributing to the disorders. In 2013, we published this work in *Molecular Psychiatry* (Thomson et al., *Mol Psych* doi 10.1038/mp.2013.68 [2013]).

To better understand the potential interaction between *DISC1* and other genes, we have been detecting variants in genes thought to interact with *DISC1.* Last year, we reported on the targeted resequencing of the *DISC1* interactome, a network of genes that converge on pathways critical for neuronal signaling and genes important in the treatment of SZ, BD, and UP. Using a custom Nimblegen solution capture of a ~13-Mb target, we resequenced in 1543 samples (using the same sample set as those sequenced in the study above) the exons, promoters, and conserved regions of 264 genes that are known to directly interact with *DISC1.* In 2013, we performed data quality control analysis to reduce the numbers of false associations in the case-control study. Samples with high missing genotypes, outlying heterozygosity rate, or discordant sex information were removed and individuals of divergent ancestry were identified. We filtered the single-nucleotide variants (SNVs) with excessive missing genotypes or a significant deviation from Hardy–Weinberg equilibrium. The final data set included 1453 samples and 284,699 SNVs. Among the SNVs with high quality, 78% have a minor allele frequency of less than 1%, and 67% of the SNVs have not been previously reported in the 1000 Genomes Project European subset. To detect the statistical associations between the single SNV and psychiatric disorders, we performed a Fisher's exact test across all SNVs on combined diagnoses: SZ, BP, and UP, respectively. To assess the associations at the genic level, we performed two gene-based association methods including the basic burden test and the sequence kernel association test. In 2014, we aim to expand the analysis to analyze epistatic interactions between genes and to further investigate and prioritize variants and genes for functional follow-up.

We also began a project to analyze the complete genomes of 39 members of the *DISC1* family in which the original translocation was found in order to search for independently segregating variants that may explain why many of the translocation carriers develop SZ or recurrent UP but others have only minor or no psychiatric diagnoses. We will then be able to compare these potential variants with the variants observed in the *DISC1* interactome. On completion of whole-genome sequencing, we were able to produce high-quality libraries with >33× mean coverage and >93% of bases covered at 20× or higher depth for each sample. The data were processed through our standard analysis pipeline that uses Burrows–Wheeler aligner (BWA) mapping (Li et al., *Bioinformatics 25:* 1754 [2009]), SAMtools (Li et al., *Bioinformatics 25:* 2078 [2009]), BamTools (Barnett et al., *Bioinformatics 27:* 1691 [2001]), and Picard for alignment processing, and QC and GATK (DePristo et al., *Nat Genet 43:* 491 [2011]) for variant calling. Recently, the variants were recalled using GATK's multisample calling feature in order to obtain more accurate genotyping across samples. In 2013, we received a grant from the NIMH to continue the study of this important locus.

In 2014, we plan to further investigate the variants and to use analysis methods and software such as the GEMINI software suite and Ingenuity Variant Analysis (www.ingenuity.com) to classify and prioritize variants (Paila et al., *PLoS Comput Biol 9:* e1003153 [2013]). We will expand the analysis from SNPs and small indels to include copy number variants (CNVs) and structural variants. In addition, we plan to perform methylation analysis to scan for differentially methylated regions and to follow up on our findings in a larger Scottish and NIMH case control set.

Exome-Based Sequencing of Parent–Offspring Trios with Schizophrenia

S.E. McCarthy, M. Kramer, Y. Berstein, E. Ghiban, E. Antoniou, W.R. McCombie [in collaboration with J. Gillis and S. Yoon, Cold Spring Harbor Laboratory; M. Mistry and P. Pavlidis, The University of British Columbia; E. Kelleher, C. O'Brien, G. Donohoe, M. Gill, D.W. Morris, and A. Corvin, Trinity College Dublin]

In collaboration with Trinity College Dublin, our data analysis of de novo mutations in 57 parent–offspring trios with schizophrenia (42 trios with no family history

of psychosis [*sporadic*] and 15 trios with a family history of psychosis [*familial*]) was finalized and submitted for publication at *Molecular Psychiatry* in December 2013. The fine-tuning of this work improved the significance of our findings summarized below.

In addition to the analysis we reported in 2012, we found in our sporadic trios that de novo mutations likely to be disrupting or damaging to protein function in genes were less tolerant to new or rare mutations ($P = 2 \times 10^{-5}$) (Petrovski et al., *PLoS Genet 9:* e1003709 [2013]). We also refined our disease ontology analysis to improve the specificity of the gene–phenotype relationships. Following this revised analysis using 72 fine-tuned *Neurocarta* (Portales-Casamar et al., *BMC Genomics 129:* doi 10.1186/1471-2164-14-129 [2013]) phenotypes (defined by 10 or more quality-filtered associated genes), the overlap between genes with de novo mutations (DNMs) and genes implicated in autism and intellectual disability remained. The overlap with autism was the greatest of DNMs in genes such as *CHD8*, *MECP2*, *AUTS2*, and *MLL2,* which supports previous genetic and epidemiological studies that suggest a shared genetic component between schizophrenia and autism.

We also improved our analysis of DNMs in potential chromatin modifier genes. First, we found a significant enrichment of DNMs in genes coding for chromatin modifiers that have been implicated in the etiology of mental illnesses. Second, we expanded the analysis to epigenetic modifying genes based on protein domain specificity and observed a significant enrichment overall of DNMs in these genes.

An important addition to this analysis was determining the consistency of our findings using data from nine exome-sequencing studies of trios with neurodevelopmental disorders, including schizophrenia ($n = 3$) and autism ($n = 4$), ID ($n = 2$), as well as six unaffected cohorts. With our data set included, an increase in nonsense mutations was observed in 40% of the neurodevelopmental data sets compared to just one control data set (de Ligt et al., *J Med 367:* 1921 [2012]; Gulsuner et al., *Cell 154:* 518 [2013]; Iossifov et al., *Neuron 74:* 285 [2012]; Neale et al., *Nature 485:* 242 [2012]; O'Roak et al., *Nature 485:* 246 [2012]; Rauch et al., *Lancet 380:* 1674 [2012]; Sanders et al., *Nature 485:* 237 [2012]; Xu et al., *Nat Genet 44:* 1365 [2012]). More than 66% of the neurodevelopmental data sets had functional de novo mutations affecting protein function in haploinsufficient genes or genes intolerant to novel mutations, in contrast to two control cohorts. No unaffected cohort had an enrichment in genes implicated in autism spectrum disorder (ASD) or intellectual disability (ID). Finally, genes involved in chromatin regulation of gene transcription were enriched in ~60% of the neurodevelopmental data sets, whereas enrichment was only observed in one control data set.

Finally, our submission included work combining the power of orthogonal and complementary genomic analysis to understand the function of genes about which little is known. Using gene expression data, genes with DNMs in our schizophrenia trios presented reduced coexpression with other genes in the prefrontal cortex, in contrast to other brain and nonbrain regions, supporting hypotheses suggesting that genes with loss-of-function mutations express lower connectivity.

We aim to expand this study of de novo variants in more trios with schizophrenia. However, given the role of chromatin remodeling and epigenetic regulation of gene transcription during brain development and in the adult brain, we aim to study how perturbations of genes implicated in chromatin remodeling affect neuronal development and function of the PVD (peripheral vascular disease) neuron in *C. elegans* and in neurons derived from patient-specific induced pluripotent stem cells, shedding light on the pathogenesis of neurodevelopmental disorders. We also aim to explore the role of chromatin remodeling in the adult mouse amygdala in response to fear conditioning, which may provide insight into the molecular mechanisms of anxiety and posttraumatic stress disorder (PTSD) that could be used to develop novel treatments for these conditions.

Investigation of Bipolar Disorder Genetics Using Exome Capture and Resequencing

J.S. Parla, S. Muller, G. Cheang, M. Kramer, E. Ghiban, S. Goodwin, W.R. McCombie [in collaboration with J. Potash, University of Iowa; P. Zandi, F. Goes, R. Karchin, and A. Chakravarti, Johns Hopkins School of Medicine]

Bipolar disorder (BP) is a complex psychiatric disease for which previous studies have failed to find significant causative variants of large effect. In 2010, we began using exome capture and next-generation sequencing to study bipolar disorder using family samples as well as case-control analysis. By 2012, we had successfully sequenced more than 60 family samples

and ~1000 case-control samples. Although this work allowed us to develop and streamline high-throughput capture and data-processing pipelines, it became quite clear that we would need a much larger sample set in order to investigate this likely polygenic phenotype. In 2013, we scaled up sequencing and brought the total samples completed for the project to 2329. This basically doubled our goal for the project and matched the output of the previous 3 years of the project combined. In 2013, we focused the analysis on variants that were predicted to be damaging by several software tools—SIFT (Ng PC1 et al., *Nucleic Acid Res 13:* 3812 [2003]), PolyPhen (Adzhubei et al., *Nat Methods 7:* 248 [2010]), and VEST (Carter et al., *BMC Genomics 14:* 1–16 [2013]), which is a tool developed by our collaborators—and that segregated with disease in the family samples. We then assessed whether there was any increased burden in case versus control samples for those genes. This analysis led us to focus on 10 possibly significant genes. We then used Sanger sequencing to genotype family members of the probands who were affected cases in the case-control sample set; however, we did not find any further evidence of segregation. In 2014, we plan to further refine our analysis techniques and to focus more on variant analysis in the larger case-control data set.

Additionally, on the analysis front, our collaborator Rachel Karchin proposed a new hybrid likelihood model to assess functional variants. The method is called BOMP (burden or mutation position test) and is a combination of a burden test with a test of the positional distribution of variants. This work was published in *PLoS Genetics* in 2013 (Chen et al., *PLoS Genet 9:* e1003224 [2013]). In 2013, we also submitted a paper to the *Journal of Human Genetics* describing our work comparing data-processing techniques, quality filters, and variant detection software tools in order to produce an accurate call set.

Functional Screening in the PVD Neuron of *C. elegans* to Unravel Neuropsychiatric Disorders

O. Mendivil Ramos, S.E. McCarthy, M. Kramer, W.R. McCombie [in collaboration with C. Aguirre-Chen and C. Hammell, Cold Spring Harbor Laboratory]

C. elegans is an important model system for the identification and functional mapping of genes involved in complex biological processes, including those of the nervous system. In particular, it has been a useful model for a variety of neuropsychiatric disorders including schizophrenia, Alzheimer's disease, and neurodegeneration. The number of neurons in the *C. elegans* nervous system is known. Moreover, most of the connections among neurons are known, as well as the lineage of every cell from a fertilized embryo to the adult animal. The PVD neuron forms several levels of well-characterized dendrites and is competent for bacterial ingestion-mediated RNA interference (RNAi). This unique feature enables functional testing for a neural developmental phenotype at high throughput.

Late in 2013, we established a collaboration with the Hammell lab at CSHL to functionally assess some of the most prominent variants that we have identified in human genetics studies as being likely associated with neuropsychiatric disorders. We assembled a list of 117 human candidate genes identified in various ongoing genetic studies and found that 47 of them had good homologs in *C. elegans.* The Hammell lab performed a preliminary screen of these genes, obtaining six phenotypes of altered branching of the PVD neuron. In 2014, both labs will be expanding this research line by extending the list of homologs to additional candidates, as well as doing controlled studies to better estimate the efficacy of this model in assessing the large number of genetic variants being identified for major psychiatric disorders.

Highly Multiplexed Targeted Resequencing of Submillion Base Genomic Targets

J.S. Parla, P. Deshpande, S. Ethe-Sayers, M. Kramer, E. Ghiban, S. Goodwin, W.R. McCombie [in collaboration with R. Karchin, Johns Hopkins School of Medicine]

Improvements in next-generation sequencing technology allow users to produce enormous amounts of sequencing data in increasingly short times, but this is predominantly directed at a large amount of sequence from each sample (such as the entire genome). There remains a significant and growing need to efficiently sample small genomic regions (on the order of 0.5 Mb) in extremely large cohorts of samples. In 2012, we worked to establish a technique using Nimblegen solution capture probes to barcode and capture highly multiplexed samples. We created a custom Nimblegen probe set targeting five genes that cover ~637 kb of the genome. We then successfully validated multiplexing up to 96 samples per capture, followed by sequencing of the 96plex set on one

Illumina HiSeq lane. We were able to achieve ~90% of target coverage at ≥20× sequence depth and ~200× mean target coverage with this method.

In 2013, we began testing methods to increase the level of sample multiplexing to take advantage of the high mean coverage that we were getting on the 96 individually barcoded samples along with increased sequence output from the Illumina instruments. We investigated sample-pooling techniques where five or 10 individuals were pooled together, and that entire pool was subsequently barcoded and sequenced up to a 96plex of 10 samples per pool (960plex). These pools were then run across three HiSeq lanes. We began testing a modified GATK pipeline (DePristo et al., *Nat Genet 43:* 491 [2011]) for variant calling in the anonymous pools. We found that we were able to detect the correct variants for the individuals within those pools to a high degree (only ~1%–3% of variants that were called in the samples when they were sequenced individually were not found in the pools on average); however, we found we also call a large number of extraneous false-positive variants with this method of ascertainment. This led as to begin studying the effect of modifying several sequence quality filters to obtain a high degree of sensitivity while reducing the number of false positives. More work is needed to refine the filters and methods used to call variants accurately in these anonymous pools.

Further investigation to explore complex pooling designs will be performed in 2014. These investigations will include simulations and bench work to evaluate the performance of various pooling designs for identifying rare and common variants. In particular, we will begin testing sample pooling using a two-dimensional matrix such that individuals will be pooled by a unique row/column location before barcoding.

Genomics of Colorectal Cancer

E. Antoniou, W.R. McCombie [in collaboration with E. Li and J. Williams, Stony Brook University]

Colorectal cancer (CRC) is the third most commonly diagnosed cancer and is the second leading cause of cancer-related deaths in the Western world. The mortality from colorectal cancer in Caucasian Americans (CAs) has been declining, but, in contrast, it has been rising in African Americans (AAs). The response to chemopreventive agents differs in terms of the biological/genetic heterogeneity of the tumors of AAs and CAs. Because chemopreventive agents exert their effect through a molecular target, this disparity would suggest differences at the genetic level. This project aims to determine the attribution of genetic and epigenetic differences to the racial/ethnic health disparity of colon cancer.

In 2012, to validate our laboratory and analysis pipelines, we used RNA-Seq on a cell line (HT29) treated with 5-aza-deoxy-cytidine. In 2013, we compared our results to the data from previous microarray studies. We published an article on this comparison, showing the superiority of the RNA-Seq technique (Xu et al., *BMC Bioinformatics 14; S1:* doi 10.1186/1471-2105-14-S9-S1 [2013]). We also used RNA-Seq to obtain gene expression measurements on 18 tumor/normal paired AA tissue samples and as many samples from CA patients. Analysis is ongoing.

In addition, we prepared 57 reduced representational bisulfite sequencing (RRBS) libraries, 52 of them from paired tumor/normal tissues. In 2014, we will prepare another 30 RRBS libraries and more RNA-Seq libraries from the same paired samples. We will then proceed with the combined analysis of gene expression and DNA methylation. Additional biopsies are also being collected on an ongoing basis at Stony Brook University Hospital.

Plant Genomics

M. Kramer, E. Antoniou, E. Ghiban, W.R. McCombie [in collaboration with M.C. Schatz, J. Stein, A. Hernandez Wences, J. Gurtowski, E. Biggers, H.Y. Lee, J. Chia, and D. Ware, Cold Spring Harbor Laboratory; L.G. Maron, M.H. Wright, and S.R. McCouch, Cornell University]

Rice (*Oryza sativa*) is one of the most important food crops in the world, especially in the developing world. Relatively little effort has been made to explore the nature of structural variation within and between subpopulations of domesticated *O. sativa*. The best way to fully understand the genomic diversity of rice is to perform whole-genome shotgun sequencing and de novo assembly. The difficulties in assembling the short reads initially provided by next-generation sequencing have resulted in efforts to sequence other strains of rice that rely on mapping the sequence read back to a reference sequence. This results in the loss of information where there are structural differences between the two strains. Often, these differences constitute biologically salient information. Recent advances in sequencing

technology and in computational approaches to sequence assembly have significantly improved the power and reliability of de novo assembly. In this project, we used these new tools to develop de novo assemblies of three divergent rice genomes representing the indica (IR64), aus (DJ123), and japonica (Nipponbare) subpopulations and to determine the extent and distribution of structural variation among them.

During the last quarter of 2012, Pacific Biosciences Inc. (PacBio) released a new polymerase and associated chemistry (P4/C2) that greatly increased the read length on this machine, up to an average of 4.5 kbp. A hardware upgrade also doubled the throughput of the instrument in mid 2013. Taking advantage of these improvements, we produced 19× coverage of the Nipponbare genome using the new P4/C2 enzyme/chemistry combination. We also obtained 28× genome coverage of longer Illumina MiSeq reads (paired end 250 bp). A new assembly of the Nipponbare genome was made using the PacBio and Illumina 250-bp reads. The N50 contig size doubled from 21 kbp to 58 kbp. Furthermore, a new error-correction algorithm for the PacBio sequences was written by Mike Schatz's group. After applying this new error-correction pipeline, the N50 contig size grew to 155 kbp. In conclusion, we can now generate a de novo assembly of a complex genome such as rice using next-generation sequencing platforms at a fraction of the cost and time that it took to make the bacterial artificial chromosome (BAC)-based rice reference genome.

In the last quarter of 2013, Pacific Biosciences introduced yet another polymerase and chemistry as well as new library preparation protocols that have doubled the average read length on this platform. We are planning to generate enough coverage of at least one rice genome with these new reagents to assemble a de novo rice genome using only PacBio sequences, with the hope of achieving even larger contig sizes.

De Novo Assembly of Yeast Genomes Using Long Insert Libraries and the Pacific Biosciences RS II Instrument

P. Deshpande, S.M. Eskipehlivan, M. Kramer, S. Goodwin, E. Antoniou, W.R. McCombie [in collaboration with J. Gurtowski, H.Y. Lee, and M.C. Schatz, Cold Spring Harbor Laboratory; C. Heiner and G. Khitrov, Pacific Bioscience]

Next-generation sequencing has proven to be an invaluable tool for the understanding of genetics. However, the relatively short reads generated by instruments such as the Illumina HiSeq pose a limitation to the de novo assembly of larger genomes. Pacific Biosciences provides an instrument that can generate reads that are thousands of bases in length. These long reads provide the architecture required for de novo assembly and also provide superior retention of structural elements important to the understanding of genetic variation.

In 2013, we began exploring the use of long read sequencing for the de novo assembly of yeast strains. We selected a large fragment (>7 kb) library from the *Saccharomyces cerevisiae* W303 genome using a BluePippin from Sage Sciences. Sequences were generated using the Pacific Biosciences RS II instrument with p5-c3 chemistry. The results yielded >75× coverage across the genome with reads in excess of 10 kb. These sequences were assembled with HGAP and the Celera Assembler (Chin et al., *Nat Methods 10:* 563 [2013]; Myers et al., *Science 287:* 2196 [2014]). The resulting contig N50 length approached one million bases, essentially only limited by the chromosome lengths of the organism. Only one chromosome, containing a very long tandem repeat, was not represented by a single contig. In 2014, we plan to continue to optimize these protocols and test them on additional yeast genomes.

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Elena Ghiban

PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware

A. Eveland, Y. Jiao, M. Karey Monaco, S. Kumari, Y.K. Lee, C. Liseron-Monfils, Z. Lu, C. Noutsos, A. Olson, S. Pasternak, F. Qiu, J. Stein, J. Thomason, P. Van Buren, B. Wang, L. Wang, S. Wei, K. Youens-Clark, L. Zhang, Z. Zhang

Our lab has two goals: (1) research into plant genomics and (2) developing tools and resources for use by the genomics research community. Our research includes a broad range of physical, statistical, and functional genomics in model plant systems and agriculturally important crop plants. We also contribute to two large-scale, multi-institutional, cyberinfrastructure collaboratives that are designed to serve broad research and educational communities. During the past year, we were joined by visiting scientist Fazhan Qi and postdoctoral researcher Bo Wang. Shiran Pasternak has advanced his career, taking a position in the industry sector.

Gramene

Y. Jiao, S. Kumari, M. Karey Monaco, A. Olson, S. Pasternak, J. Stein, J. Thomason, S. Wei, K. Youens-Clark [in collaboration with P. Jaiswal, Oregon State University; P. Kersey and H. Parkinson, EMBL–European Bioinformatics Institute; L. Stein, Ontario Institute of Cancer Research; C. Taylor, American Society of Plant Biologists]

The Gramene project provides online reference resources for plant genomes and curated pathways to aid functional genomics research in crops and model plant species. Our website, www.gramene.org, allows the study of gene function by combining genome annotation and experimental data with cross-species comparisons. This past year witnessed remarkable growth of the website as the project accomplished several major milestones culminating in our 40th release since the inception of this project. Highlights include the addition of six new species, the premiere of the Plant Reactome database, and a total redesign of Gramene webpages and search functions.

The Gramene team develops comparative genomics databases in collaboration with the Ensembl Plants project at the European Bioinformatics Institute (EBI). Thanks to this teamwork, we added six new complete reference genomes to the collection, bringing the total to 28. Perhaps the most significant of these was the genome of bread wheat, *Triticum aestivum*, released by the International Wheat Genome Sequencing Consortium. This hexaploid genome is the largest (~17 Gbp) and most complex angiosperm genome ever sequenced. The first draft, although gene-rich, is still a highly fragmented assembly; nevertheless, its addition to Gramene completes the triumvirate of major cereal grains in our collection: wheat, rice, and maize. These three crops constitute 90% of world-wide grain production and are keystones to future agricultural sustainability. Bread wheat's unusually large genome can be attributed in part to allohexaploidy (AABBDD), which arose during domestication. Understanding the evolution and interaction among subgenomes will be aided greatly by study of its diploid progenitors. Two of these, *Triticum uartu* (AA) and *Aegilops tauschii* (DD), were also added this past year to the Gramene/Ensembl Plants collection, following their release by the Beijing Genomics Institute (BGI). These, along with the barley genome (*Hordeum vulgare*), make Gramene a preeminent site for study of Triticeae genomes.

Gramene continues to build a genus-level phylogenomics resource for rice and related *Oryza* species. Rice emerged ~15 million years ago. The *Oryza* genus includes two cultivated species (Asian and African rice) and 21 wild species adapted to a broad range of tropical and subtropical habitats around the world. The genomics research wishing to exploit such diversity for crop improvement is also establishing *Oryza* as a model system for genus-level study of trait evolution, speciation, and domestication. Working with the National Science Foundation (NSF)-funded Oryza Genome Evolution (OGE) project (principal investigator: Rod Wing, University of Arizona) and with the international consortium known as I-OMAP, Gramene will soon host high-quality genome assemblies for more than half of these species. Last year,

Gramene added complete reference assemblies for two new species and will include six additional genomes in the coming release, bringing the total to 12. One of the challenges of comparative genomics research is that different genome projects apply different protocols to identify genes, leading to methodological bias that confounds comparative analysis. To remedy this, Gramene staff worked with the OGE project to develop a consistent annotation protocol (using the MAKER-P pipeline) that was applied both to the new genomes and to the *Oryza* genomes previously sequenced and annotated by other projects. Gramene will continue to support both sets of annotations by creating a new website to represent I-OMAP genomes and phylogenetic trees.

PLANT GENOME RESEARCH

In the last decade, the decoding of complete plant genomes has helped scientists understand plant function and evolution and how to alter economically important traits. Many disciplines are required to generate reference genomes. This starts with laboratory scientists who generate the raw sequence data and goes to computational biologists and bioinformaticians, such as those in our lab, who interpret the output. This interpretation includes the assembly of raw sequence reads into overlapping segments ("contigs") that are used to create a scaffold to discern 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, such as in web-based platforms like 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 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 or 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 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 underlying genome sequence. Epigenetic mechanisms include modification of DNA by methylation and various forms of histone modification that can cause changes in gene expression and other phenotypes. Both of these modification types can be studied with new sequence technologies and analysis methods.

Updating the Maize B73 Reference Genome and Developing Pan-Genome Variation Pipelines

This work was done in collaboration with J. Glaubitz, Cornell University; E. Buckler, Cornell University; R. Fulton, Washington University; R. Wilson, Washington University; and the Maize Genome Sequencing Consortium. Work continues on refining the assembly and annotation of the maize B73 reference sequence. The third version ("RefGen_v3") of the B73 assembly and annotations is hosted on Gramene and has been deposited in GenBank along with contigs assembled from Roche/454 sequencing of a whole-genome shotgun library. RefGen_v3 incorporates some of these contigs in order to increase coverage of missing gene space. The contigs were selected based on alignment to FLcDNA sequences and inserted into gaps in the RefGen_v2 assembly guided by a genetic map combined with synteny to rice and sorghum. Approximately 500 genes were added or improved with this method, and many unplaced physical map contigs were also anchored on the basis of these maps. The RefGen_v3 assembly was released following acceptance by GenBank.

Work on RefGen_v4 is under way. To improve the maize genome sequence, we are adding additional depth to the existing capillary sequence using the Illumina 2000 platform, as well as generating long reads with PacBio to span repeats. We have resequenced 17,173 bacterial artificial chromosomes (BACs) that constituted the minimal BAC tiling path from the original B73 sequencing project by pooling the BACs in pools of 96 and are generating Illumina data to greater than 150× coverage per clone. This increased depth, density of read pairs, and differential

bias compared to capillary sequencing will serve as an excellent resource to further improve the maize sequence. Long reads from PacBio will be used to improve the order and orientation of assembled contigs and close gaps wherever possible. These data, once completed, will be made available through public databases before completion of the RefGen_v4 assembly.

In *Zea mays*, it is estimated that only 50% of genomic content is held in common between lines due to tremendous haplotype diversity. A single reference assembly for maize cannot serve as a sufficient backbone to capture and describe this variation and is a limiting factor in understanding the genetic variation controlling traits. The maize pan-genome under development will be composed of the B73 reference assembly and novel sequences from other maize inbred lines. Our initial efforts are to identify novel haplotypes among a broad sampling of sequenced germplasms by conducting whole-genome assembly of 30× Illumina reads. At this depth, high-repeat content in maize will challenge existing methods for de novo assembly. Simulations using the ALLPATHS-LG recipe to assemble 30× reads sampled from the B73 reference resulted in ~82% coverage of annotated genes. We are using sequence data for the inbred B97 to further prototype pipeline construction. Because different assembly algorithms have different advantages, we have adopted a meta-assembly strategy. Using this strategy, ~30% of the genome could be assembled with ~72% of genic space covered in scaffolds. After aligning to the BAC-based B73 reference, ~19.78 Mb of novel sequences was identified. We also used a genetic mapping method to anchor novel sequences relative to the B73 physical map. The accuracy rate of this genetic mapping method was tested by anchoring 1000 B73 genes, and the results demonstrated 77.6% accuracy. As for B73, PacBio long reads will be generated and used for scaffolding and gap closing.

Discovery and Application of Epigenetic Variation in Sorghum and Maize

Z. Lu, A. Olson [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 Pioneer; D. Dugas, R. Klein, and P. Klein, USDA/Texas A&M University]

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. In collaboration with the Martienssen lab and DuPont Pioneer, we sequenced the methylome of two maize inbred lines, B73 and Mo17. The genomic DNA is treated with bisulfite, which converts 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 the 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 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.

In *Sorghum*, we used shallow sequencing (2×–4×) of bisulfite-treated root tissues in order to identify regions of open chromatin and patterns of methylation associated with high-confidence gene models. These patterns, together with measures of gene expression and sequence conservation, were used to train a classifier to predict functional gene models. A new set of gene models was produced using the Gramene/EnsEMBL GeneBuilder by combining transcript fragments assembled from RNA-sequencing data with alignments of protein, expressed sequence tags (ESTs), and cDNA sequences from sorghum and other monocots. The classifier, trained on Sbi1.4 gene models, was run on the new set of gene models and predicted a total of 56,128 functional genes and transcriptionally active regions in sorghum.

Characterization and Analysis of Core Promoter Elements in Plant Genomes

S. Kumari

Where and when a plant will express or transcribe a gene is controlled by information in the plant genome and is influenced by environmental signals. Transcription

initiation involves the recruitment of the basal transcription factors to the core promoter elements (CPEs) of genes. Very little is known about CPEs in plants, and this study offers a new insight in the field. To better understand the core promoter architecture in plant genomes, we studied a comprehensive set of known CPEs in various monocot and dicot plant genomes.

We developed high-throughput in silico methods for motif prediction using the publicly available CREAD (comprehensive regulatory element analysis and discovery) suite of tools. Our computational pipeline systematically identified transcription regulation motifs at the whole-genome level including TATA-box, CCAAT, INR, BRE, GC-box, DPE, MTE, and Y-Patch motifs containing genes across eight plant genomes. This is the first large-scale genome-wide computational study that has compared and contrasted the distribution profiles of known CPEs across four monocots (*Brachypodium distachyon, Oryza sativa japonica, Sorghum bicolor, Zea mays*) and four dicots (*Arabidopsis thaliana, Populus trichocarpa, Vitis vinifera, Glycine max*). The relative abundance signal of CPEs with respect to transcription start sites (TSSs) was found to be conserved within monocots and dicots with slight differences across monocots and dicots. The TATA-box motif was found to be present in 16%–22% of the plant promoters, whereas the GC-box, XCPE1, and Y-patch motifs were found to be the most prevalent CPEs across all eight genomes.

The structural properties in the regulatory part of genomes distinctly differ from those of the nonregulatory part. The core promoter region's free-energy profiles (Fig. 1) segregate into two distinct clusters: one clustering monocots together and the other clustering dicots together. Free-energy profiles have the potential to be used for delineating the promoter region as well as for computational TSS identification. It could help build better computational models for predicting the TSS in the promoter region, which remains a challenging problem.

We classified core promoters into three types (TATA-containing, TATA-less, and Core-less) and looked at the functional enrichment of genes associated with these types. TATA-containing genes showed significant overrepresentation of biological processes that included response to abiotic and biotic stress, hormonal stimuli, and regulation of carbohydrate and nucleic acid metabolic processes. TATA-less genes were mainly involved in housekeeping, including transferase activities, hydrolase activities, and various nucleotide-related binding activities. These genes were mainly enriched in biological processes related to nitrogen and phosphorous metabolism, which are key genes involved in plant-yield-associated traits. The Core-less genes showed evidence of unique enrichment in genes involved in ATP binding, signal transduction activities, apoptosis, etc. Our study expands the CPE repertoire in plants, providing impetus for future genome annotation projects and inspiring research efforts aimed at better understanding transcriptional regulation mechanisms and the relationship for crop yield improvement.

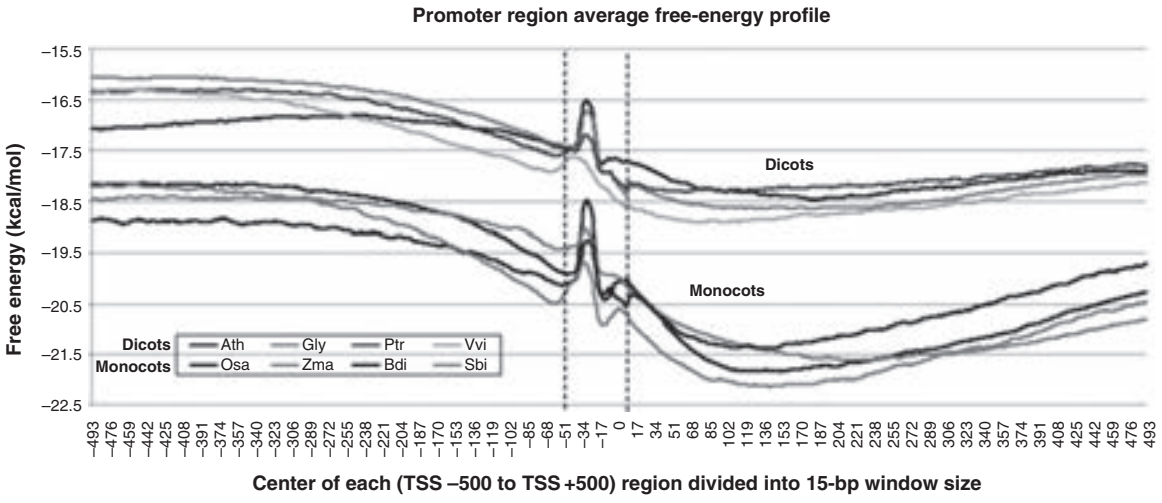

Figure 1. Free-energy profile of the promoter region genes from eight plant genomes.

PLANT SYSTEMS BIOLOGY

Exploring the Gene Regulatory Network Guiding Arabidopsis Stele miRNA Expression

L. Zhang, Y. Koung Lee, C. Liseron-Monfils, C. Noutsos, B. Wang [in collaboration with S. Brady, University of California, Davis, and DuPont Pioneer.]

Plant roots not only serve to physically anchor plants to the soil but are also responsible for uptake of water and critical nutrients; therefore, they must respond quickly to various environmental stresses such as drought, waterlogging, and heavy metal pollution. microRNAs (miRNAs) have a central role in plant development and response to environmental stress. We are interested in studying the gene regulatory network (GRN) that regulates miRNA expression in the root. Most miRNAs are transcribed by RNA polymerase II, and their transcription process is regulated by transcription factors (TFs). To systematically resolve points of cross-talk among TFs, miRNAs, and their targets in a comprehensive GRN, we make use of a gene-centered yeast one-hybrid (Y1H) experimental system that allows us to monitor protein binding to DNA in yeast. We have extended the library that initially focused on transcription factors from the root stele (657), the central part of the root important for nutrient transfer, to the complete root (952). Using this system, we screened 172 promoters of stele-expressed miRNAs, their targets, and some highly connected TFs. We have obtained 3768 protein–DNA interactions (PDIs). The resulting network was analyzed with publicly available spatiotemporal expression data in the root that allowed us to examine whether the TFs act as activators or inhibitors of expression. To validate the PDIs found in our yeast work, we screened more than 250 genetic insertion mutants from *Arabidopsis* and obtained 80 lines for TFs, miRNAs, and miRNA targets in our network. From these, we characterized their molecular phenotype by quantifying TF expression. Our results showed that perturbation in planta of ~80% of TFs and 70% of miRNAs tested displayed molecular phenotypes in the *Arabidopsis* root.

In addition to molecular phenotypes, the plants were also scored for observable morphological differences, and two mutants with a short-root phenotype were identified. To understand the genes that were bound by one transcription factor, we used a chromatin immunoprecipitation sequencing (ChIP-Seq) approach that allows us to identify plant DNA that is bound by a transcription factor. Using this approach, we identified both genic and intergenic regions bound by the transcription factor and used this to predict a DNA motif that is likely bound by the transcription factor. Further genetic investigation of this transcription factor suggested that it may be involved in stem cell maintenance in the root.

We have begun to use the network to predict genes that may be important in development based on the topology of the network. Within the network, some genes are more highly connected, which suggested that these genes may have an important role in the network. The zinc finger homeodomain (ZF-HD) TFs were found to be highly connected in our network. These genes are conserved across the plant kingdom. We have managed to identify them in more than 300 species. The number of ZF-HD varies from species to species. In *Arabidopsis,* there are a total of 16 members. Genetic analysis of these TF families indicates that their functions are not restricted to the root and that they are important in flower development. Although our network was focused on root-expressed genes, more than 80% of these genes are expressed in other tissues and will be more broadly applicable beyond root development and response to stress. The GRN provides a framework for modeling adaptive responses to environmental conditions at the whole-plant level. We can use this network to identify candidate genes for improving germplasm that can withstand more detrimental conditions, thus addressing global food security and growing demand for renewable energy resources.

Developmental Networks Controlling Inflorescence Architecture in Maize

A.L. Eveland, S. Kumari, C. Liseron, Y. Jiao [in collaboration with D. Jackson, Cold Spring Harbor Laboratory; Z. Xin and J. Burke, USDA Agricultural Research Service]

The goal of this work is to integrate genetics and genomics data sets to find molecular networks that influence the morphology (architecture) of maize inflorescences (flowers). 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 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.

The primary objectives for this project included the following: (1) Establish a comprehensive pipeline for mRNA-Seq and ChIP-Seq data analysis in maize by evaluating and optimizing available software for mapping and quantification. This also included 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 from this included identification of candidate genes, specifically developmentally regulated TFs, and novel genes of unknown function that are potentially involved in stem-cell maintenance and determinacy. We continue to examine coexpression clusters for enrichment of functional processes and *cis*-regulatory motifs that lie within proximity of 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 addition to maize, we have begun work on sorghum, which is an important emergent bioenergy crop used for human consumption in sub-Sahara Africa. We are using next-generation sequencing approaches to identify the single-nucleotide mutations that are associated with an increase in seed of the sorghum plants in a sorghum EMS (ethylmethanesulfonate) population. Using this approach, we have identified two genes that can change the structure of flowers and generate more seeds. We intend to apply this strategy to perform a large-scale sorghum mutant study to support candidate genes associated with developmental traits in the roots, shoots, and flowers.

In the next phase, we will further prioritize candidates from this work by overlaying the *Arabidopsis* regulatory network information (see previous section). The resulting hypotheses can be tested in *Arabidopsis*, for example, for 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 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.

CYBERINFRASTRUCTURE PROJECTS

The iPlant Collaborative

L. Wang, J. Lu, C. Noutsos, J. Stein, Y.K. Lee

This project was done in collaboration with Cold Spring Harbor Laboratory and employs more than 100 staff. It is headquartered at the University of Arizona under the direction of principal investigator Stephen Goff and director Dan Stanzione. Dozens of collaborators are located at more than 20 institutions.

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 the first five-year grant, the iPlant Collaborative has made extensive

progress toward meeting these goals and has been recommended for renewal for another five years. 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 scientists and educators. Within the Ware lab, these platforms include the Discovery Environment (DE), Atmosphere, and the Taxonomic Name Resolution Service (TNRS).

The DE is the most visible portal to iPlant tools and services. This web-based platform supports an "app store" model of user-extensible tools, automated workflows, and data storage. Users can take advantage of existing tools integrated by iPlant staff and user community or add their own tools to use privately or share. Users may not be aware that the underlying infrastructure provides access to iPlant's massive data store at the University of Arizona and the Texas Advanced Computing Center (TACC). Computationally intensive tasks are handled by supercomputers located at TACC and other centers within the Extreme Science and Engineering Discovery Environment (XSEDE). So far, well over 400 tools have been integrated into the DE that enable a broad range of research activities, including genome/transcriptome assembly, annotation, RNA-Seq quantification, variant detection, genome-wide association studies (GWAS), and phylogenetics. Members of our lab have had important roles in contributing to workflow design, tool integration, validation, science tutorial, and documentation.

Atmosphere is iPlant's configurable and cloud-enabled computational resource for 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 precustomized. Users can also create their own applications and environments as VMs and share with others via Atmosphere. 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 various binary tools and R statistics packages used in ecology and genetics research and for plotting complex data in graphs.

The success of genome research depends on our ability to accurately assemble, annotate, and derive meaning from sequence data; however, extremes of genome size, polyploidy, diversity, and repeat content push the limits on current algorithms, expertise, and computational power needed by today's researchers. In response, iPlant is fostering a community effort to identify best practices and state-of-the-art tools, install and optimize their performance on the nation's most powerful supercomputers, and make these available as a free online resource. During the last two years, the iPlant Discovery Environment has matured to provide a comprehensive set of tools and services for sequence handling, performing read alignments, RNA-Seq profiling, and de novo genome and transcriptome assembly. To extend these capabilities, we are working to incorporate MAKER-P, a standardized, portable, and easy-to-use plant-genome annotation engine with built-in methods for quality control. As part of this effort, MAKER-P was specifically optimized to take advantage of the parallel computing environment of the TACC Lonestar cluster and is now a supported module. Performance testing showed that MAKER-P can perform high-quality, full-fledged annotation pipelines on even the largest plant genomes in a matter of hours. Incorporation of this resource into the DE fits into an overall strategy that includes downstream functional annotation of protein-coding genes and visualization. MAKER-P is currently available for use as an Atmosphere image.

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 any published name; furthermore, 5%–20% of published names may be synonymous. Correcting and harmonizing taxonomy are usually the time-consuming and ad hoc responsibility of the individual researcher. The TNRS tool overcomes this barrier, enabling higher-quality comparative biodiversity science. The 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 and is described in a recent publication in *BMC Bioinformatics*.

A major mission of iPlant is to promote adoption of the cyberinfrastructure through training workshops and outreach at academic institutions and scientific meetings. In 2013, members of the Ware lab participated as instructors in several tools and services workshops focused on transcriptomics, annotation, GWAS, and phylogenetics using the DE, Atmosphere, and Data Store platforms.

KBase, the Department of Energy Systems Biology Knowledgebase

S. Kumari, S. Pasternak, J. Thomason

This project was done in collaboration with the Department of Energy national laboratories and is led by principal investigator Adam Arkin of Lawrence Berkeley National Laboratory (LBNL), with co-principal investigators Rick Stevens, Argonne National Laboratory (ANL); Robert Cottingham, Oak Ridge National Laboratory (ORNL); and Sergei Maslov, Brookhaven National Laboratory (BNL). In addition to Doreen Ware at CSHL, participating investigators included Mike Schatz, CSHL; Pamela Ronald, University of California, Davis; Matthew DeJongh, Hope College, Michigan; Gary Olsen, University of Illinois, Urbana-Champaign; and Mark Gerstein, Yale University.

The DOE Systems Biology Knowledgebase (KBase, www.kbase.us) has two central goals. The scientific goal is to produce predictive models, reference data sets and analytical tools, and to demonstrate their utility in DOE biological research relating to bioenergy, the carbon cycle, and the study of subsurface microbial communities. The operational goal is to create the integrated software and hardware infrastructure needed to support the creation, maintenance, and use of predictive models and methods in the study of microbes, microbial communities, and plants.

Doreen Ware serves as the Plants Science Team Lead for KBase. In addition to providing domain expertise in plant genomics, members of the Ware lab are making significant contributions to software development for this project. The Plant component of KBase allows users to model genotype-to-phenotype relationships using metabolic and functional networks as well as phenotype measurements and high-throughput experiment data. It also supports the reconstruction of new metabolic and functional networks based on expression profiles, protein–DNA, and protein–protein interactions. To accomplish this, we have provided interactive, data-driven analysis and exploration across multiple experiments and diverse data types. We have built narratives that can capture analyses, including rich annotations, visualization widgets, reusable workflows, and custom scripts.

Through KBase resources and infrastructure, users have access to workflows for analyzing data such as those from genotyping assays and expression profiles as well as metabolic models. Users can integrate these with public resources for plant genomes. Using the KBase application programming interface (API), users can create complex queries across disparate data sources and types.

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Christophe Liseron-Monfils

Gurinder S. "Mickey" Atwal and colleagues are applying insights from the physical and computational sciences to the study of population genetics and human disease. The Atwal lab 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 of *p53*, a master tumor suppressor gene, in female fertility and furthered our understanding of how complex gene networks evolve. The lab has analyzed the comparative genomics and physical organization of cancer-related genes and their role in mediating tumorigenesis across numerous tissue types. Recently, they have begun to focus efforts on understanding cancer genome evolution on shorter time scales by analyzing nucleotide sequences from single cells.

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. He 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.

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 recurrent, aberrant genomic loci observed 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 discover such "cores" and has used it to analyze multiple genome data sets in breast, liver, ovarian, and prostate 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 these novel statistical tools to the latest generation of experimental data, which have characterized tumor samples down to the level of single cells. By interpreting single-cell genomes, he and colleagues seek to learn how specific tumors evolve and how cancer cells migrate to invade adjacent tissues and metastasize.

There is increasing evidence that rare and unique mutations have a significant role in the etiology of many diseases such as autism, congenital heart disease, and cancer. **Dan Levy**'s group develops algorithms to identify these mutations from large, high-throughput data sets comprising thousands of nuclear families. After earlier working with high-resolution CGH arrays, Levy's group now uses targeted sequence data. Levy has developed methods for identifying de novo mutations (i.e., those seen in a child but not in his or her parents) by simultaneously genotyping the entire family; the team is currently focused on building algorithms to detect copy-number variants and multiscale genomic rearrangements. Although their copy-number methods are based on "read" density, there are classes of mutations that require analysis at the level of the read. Thus, they are developing algorithms to identify insertions, deletions, inversions, transpositions, and other complex events. Other projects in the Levy lab include analysis of single-cell RNA, phylogenetic reconstruction from sparse data sets, and disentangling haplotypes from sperm and subgenomic sequence data.

Michael Schatz is a computational biologist and an expert at 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, probe sequence variations, and 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. Consequently, 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. In a recent breakthrough, Schatz was able to create a hybrid software-based solution to eliminate errors in so-called third-generation sequencing. This makes it remarkably easier to compile, align, and analyze full-genome sequences.

QUANTITATIVE BIOLOGY

G.S. Atwal R. Aboukhalil R. Korff
Y. Cai W. Liao
B. Fendler

Fueled by data generated from recent technological developments in DNA sequencing, our lab is primarily focused on population genetics, cancer biology, and high-performance computing. We often tackle scientific questions analytically and computationally by invoking theoretical concepts from statistical physics and machine learning.

Previous work in our lab, in collaboration with colleagues at the Cancer Institute of New Jersey and Weill Cornell Medical College, has established the association between single-nucleotide polymorphisms (SNPs) in the p53 tumor suppressor pathway and female infertility in mice and humans. This hypothesis was first generated through computational investigations of haplotypic diversity and positive selection in genes in the TP53 pathway where we detected signatures of recent positive selection in SNPs present at high frequencies in various human populations. Selected alleles in SNPs in *LIF, Mdm2, Mdm4*, and *Hausp* genes, each of which lies in the p53 molecular network, were found to be enriched in women undergoing IVF (in vitro fertilization) treatment. Moreover, some of these SNPs have been demonstrated to be associated with estrogen-driven cancer risk, highlighting the pleiotropic character of genetic variants in the p53 pathway. More recently, we have begun to investigate the association of SNPs in p63 and p73 and initial results are encouraging, although we await an increase in sample numbers before the results can be deemed to be statistically significant.

Following up on these earlier investigations, we have begun exploring the contribution of multiple alleles from both a population genetics and human disease perspective. In addition, we have begun refocusing efforts applying the tools of population genetics to understand single-cell evolution from tumors. This systems biology approach has led to the development of new computational tools, addressing the vast complexity of genomics data. In addition, our lab has continued research in the use of information theory and other machine learning tools in addressing the tsunami of data generated by next-generation sequencing.

Suyash Shringapure, a former visiting researcher from Carnegie Mellon, now at Stanford University, continues to collaborate with us and Bud Mishra (New York University). Bernard Fendler, a postdoctoral associate with training in theoretical physics, obtained a computational biologist position at Brigham and Women's Hospital. Willey Liao and Ying Cai are graduate students from the Applied Mathematics and Statistics department at Stony Brook. Willey Liao graduated in May and took a bioinformatician position at the New York Genome Center. Ricki Korff was an URP student during the summer of 2013 and continues to work with us remotely. Robert Aboukhalil is a graduate student from the Watson School. In addition, two first-year students from the Watson School graduate program, Talitha Forcier and Abram Handly-Santana, joined the lab for rotation projects.

Web Tool for Multispecies Gene Colocalization Analysis

R. Aboukhalil, B. Fendler [with technical assistance from P. Andrews, Cold Spring Harbor Laboratory]

The evolutionary pressures that underlie the large-scale functional organization of the genome are not well understood in eukaryotes. Recent evidence suggests that functionally similar genes may colocalize (cluster) in the eukaryotic genome, suggesting the role of chromatin-level gene regulation in shaping the physical distribution of coordinated genes. However, few of the bioinformatic tools currently available allow for a systematic study of gene colocalization across several evolutionarily distant species. Furthermore, most tools require the user to input manually curated lists of gene position information, DNA sequence, or gene homology relations between species. With the growing number of sequenced genomes, there is a need to provide new comparative genomics

tools that can address the analysis of multispecies gene colocalization. We built Kerfuffle, a web tool designed to help discover, visualize, and quantify the physical organization of genomes by identifying significant gene colocalization and conservation across the assembled genomes of available species (currently up to 47, from humans to worms). This is the first such web tool from the newly installed web server set up in the Quantitative Biology group. Kerfuffle only requires the user to specify a list of human genes and the names of other species of interest. Without further input from the user, the software queries the e!Ensembl BioMart server to obtain positional information and discovers homology relations in all genes and species specified. Using this information, Kerfuffle performs a multispecies clustering analysis, presents downloadable lists of clustered genes, performs Monte Carlo statistical significance calculations, estimates how conserved gene clusters are across species, plots histograms and interactive graphs, allows users to save their queries, and generates a downloadable visualization of the clusters using Circos software. These analyses may be used to further explore the functional roles of gene clusters by interrogating the enriched molecular pathways associated with each cluster. Kerfuffle can be found at http://www.atwallab.org/kerfuffle/ and runs on a new dedicated web server that we purposed for the entire Quantitative Biology program.

Parametric Inference in the Large Data Limit Using Maximally Informative Models

[This work was done in collaboration with J. Kinney, Cold Spring Harbor Laboratory]

Motivated by data-rich experiments in transcriptional regulation and sensory neuroscience, we consider the following general problem in statistical inference: When exposed to a high-dimensional signal S, a system of interest computes a representation R of that signal, which is then observed through a noisy measurement M. From a large number of signals and measurements, we wish to infer the "filter" that maps S to R. However, the standard method for solving such problems, likelihood-based inference, requires perfect a priori knowledge of the "noise function" mapping R to M. In practice, such noise functions are usually known only approximately, if at all, and using an incorrect noise function will typically bias the inferred filter. Here, we show that in the large data limit, this need for a precharacterized noise function can be circumvented by searching for filters that instead maximize the mutual information I[M;R] between observed measurements and predicted representations. Moreover, if the correct filter lies within the space of filters being explored, maximizing mutual information becomes equivalent to simultaneously maximizing every dependence measure that satisfies the data processing inequality. It is important to note that maximizing mutual information will typically leave a small number of directions in parameter space unconstrained. We term these directions "diffeomorphic modes" and present an equation that allows these modes to be derived systematically. The presence of diffeomorphic modes reflects a fundamental and nontrivial substructure within parameter space, one that is obscured by standard likelihood-based inference.

Equitability, Mutual Information, and the Maximal Information Coefficient

[This work was done in collaboration with J. Kinney, Cold Spring Harbor Laboratory]

How should one quantify the strength of association between two random variables without bias for relationships of a specific form? Despite its conceptual simplicity, this notion of statistical "equitability" has yet to receive a definitive mathematical formalization. Here, we argue that equitability is properly formalized by a self-consistency condition closely related to data processing inequality. Mutual information, a fundamental quantity in information theory, is shown to satisfy this equitability criterion. These findings are at odds with the recent work of Reshef et al. (*Science 334:* 1518 [2011]), which proposed an alternative definition of equitability and introduced a new statistic, the "maximal information coefficient" (MIC), said to satisfy equitability in contradistinction to mutual information. These conclusions, however, were supported only with limited simulation evidence, not with mathematical arguments. Upon revisiting these claims, we proved that the mathematical definition of equitability proposed by Reshef et al. cannot be satisfied by any (nontrivial) dependence measure. We also identify artifacts in the reported simulation evidence. When these artifacts are removed, estimates of mutual information are found to be more equitable than estimates of MIC. Mutual information is also observed to

have consistently higher statistical power than MIC. We conclude that estimating mutual information provides a natural (and often practical) way to equitably quantify statistical associations in large data sets.

Modeling Morphogenesis in Leaf Polarity and tasiRNA Regulation of ARF in Moss

[This work was done in collaboration with the Timmermans lab, Cold Spring Harbor Laboratory]

Adaxial–abaxial polarity is essential for leaf functioning in plants. The polarity is sharply defined by a precise establishment of gene expression patterns with cells across the adaxial–abaxial axis. Previous work in the Timmermans lab has shown that two short RNAs—miR166 and *trans*-acting short interfering (tasiR-ARF) may act as morphogens in establishing leaf polarity. However, it is not clear if these two opposite gradients of small RNA are sufficient to explain the sharp spatial expression pattern in leaves. We first modeled the dynamic behavior of the morphogen using a minimal network of the relevant genes, whereby tasiR-ARF inhibits ARF3/4 and miR166 inhibits HD-ZIPIII. A fixed-point analysis indicated that the system is stable with respect to small perturbations, although the influence of stochastic noise has yet to be determined. We next investigated the positional information carried by the morphogens using tools from information theory in an effort to determine how a cell optimally predicts its position in the adaxial–abaxial axis using the two opposing morphogens. The analysis from simulations of the stochastic concentration gradients indicated that the two RNA gradients were indeed sufficient to convey enough information to induce two cell fates. Further work remains to supplement the simulations with actual RNA-Seq data from each cell layer.

Analysis of *Physcomitrella patens* mutants perturbed in tasiRNA biogenesis reveals defects in the filamentous stage of moss development. These defects are the result of a spatial misregulation of the evolutionarily conserved targets of the tasiRNA pathway, the auxin response factors (ARFs). The similarity between these phenotypes and those of moss mutants defective in auxin signaling suggest that tasiRNAs may modulate plants' response to this ancient hormone. Yevgeniy Plavskin modeled the effect of tasiRNA regulation of ARF levels on the output of the auxin response gene regulatory network in the context of network topology differences identified between *Arabidopsis* and *Physcomitrella*. The model predicts that tasiRNAs sensitize cells to auxin and may have a complex effect on the robustness of the auxin response to noisy signals. This suggests that the robust yet sensitive auxin response that results from the structure of the auxin response network and its regulation by tasiRNAs may have favored its repeated cooption over the course of evolution.

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DE NOVO MUTATION IN AUTISM

I. Iossifov E. Dalkic

We study the genetics of common diseases in humans using two main tools: next-generation sequencing and molecular networks representing functional relationship among genetic loci. These tools in combination enable the large-scale studies necessary for furthering our understanding of the complex etiology of disorders such as autism, bipolar disorder, and cancer. We focus on both developing new methods (for sequence analysis and for building and using molecular networks) and applying them to specific biomedical problems.

Exome Sequencing Reveals De Novo Gene Disruptions in Children on the Autistic Spectrum

The genetic component of autism can be transmitted or acquired through de novo mutation. Previous studies have focused on large-scale de novo copy-number events, either deletions or duplications, and have identified a large number of autism candidate genes. Because copy-number events often span many genes, discerning which of the genes in the target region contribute to the disorder requires complex network analysis and inference. In contrast, with high-throughput DNA sequencing, we can readily search for de novo single-nucleotide and small-insertion or deletion mutations that affect a single gene. Such mutation is fairly common, ~100 new mutations per child, but with only a few—on the order of one per child—falling in coding regions.

We are collaborating in an ongoing large project for sequencing the exomes of 2800 families from the Simons Simplex Collection (SSC). Our preliminary analysis (Iossifov et al., *Neuron 74:* 285 [2012]) of ~350 of these families, as well as the reports of three other groups, demonstrated the power of identifying de novo mutations through exome sequencing to implicate autism genes. Among the major results were (1) strong evidence for the role of likely gene-disrupting (LGD) mutations (nonsense, splice site, and frame shifts), with affecteds having twice as many LGDs compared to unaffected siblings; (2) the identification of 127 de novo LGD mutations across the four reports with five genes (*CHD8*, *DYRK1A*, *KATNAL2*, *POGZ*, *SCN2A*) having two de novo mutations in unrelated individuals ("double hits"); (3) estimation of a total number of ~400 autism target genes, with a prediction of ~100 double hits after the whole set of 2800 families has been processed; and (4) the discovery of a strong association between the target LGD mutations in autism and in vivo targets of the RNA-binding translational regulator FMRP (encoded by *FMR1*, which results in fragile X syndrome when silenced or mutated).

In the follow-up meta-analysis of four published whole-exome sequencing data sets in autism (Ronemus et al. 2014), we more than doubled the number of recurrently hit genes, clearly demonstrated the role of missense mutations and the increased rate of de novo LGD mutations in girls with autism compared to boys with autism, and established a very important relation between de novo LGDs and IQ.

In our work with whole-exome sequencing of the SSC, the project comprises 2800 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 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 the whole-exome sequencing for large numbers 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.

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IN SILICO CANCER GENOMICS

A. Krasnitz M. McGillivray 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 TCGA and ICGC, and with the advent of new experimental methodologies, especially the next-generation sequencing and single-cell genomics. We see our goal as channeling this flood of data into a number of clinically relevant applications. These include pinpointing and prioritizing targets for functional analysis; discovery of genomic markers for clinical outcome and molecular classification of cancer; elucidating the clonal structure of tumors and its relation to progression, invasion, metastasis, and response to treatment. All of our work is done in close coordination with experimental studies performed by the Wigler, Hicks, Powers, Lowe (presently at Memorial Sloan-Kettering Cancer Center), and Stillman laboratories at CSHL.

Recurrence Analysis of Genomic Interval Data

Large collections of intervals commonly arise in high-throughput genomics. For example, DNA copy-number analysis yields intervals of the genome corresponding to gains or losses of DNA segments. Likewise, chromatin structure is often reported as intervals of the genome. In such cases, a common goal is inference of contiguous genomic target regions, which, under certain model assumptions, generates the observed patterns in the data. We call such target regions "cores." The typical evidence for cores is the presence of "recurrent" observations, suitably defined.

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. Furthermore, differences in core patterns among individual tumor cells may be used to explore genomic heterogeneity of cancer and examine genealogical relationships among tumor cell populations.

We designed and implemented a method for identifying cores in large collections of genomic interval data. A descriptive name for the method is cores of recurrent events (CORE). Central to CORE is the notion of explanatory power. We say that a core explains an event and we 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. CORE is now available to the community via the Comprehensive R Archive Network (CRAN).

Tumor Cell Population Structure

Study of genomic and transcriptional properties of individual cells is now the focus of multiple research laboratories around the world. In application to cancer, this line of research has revealed the genomic complexity of the disease and the presence of multiple genealogically related cell populations in a tumor. Detailed knowledge of the clonal structure of a cancer potentially is of high clinical value: Multiplicity of clones or of lesions in most advanced clones is a possible measure of progression; spatial pattern of clone dispersal in a tumor may signal elevated

propensity to invade; lesions observed in individual clones but not in the bulk tissue may point to targets for therapy. DNA copy-number profiling of cells from low-coverage sequencing is an accurate, economically feasible technological approach to the study of cancer subpopulation structure. Novel multiplex sequencing techniques, developed by the Wigler lab at CSHL, permit simultaneous sequencing of dozens of single-cell DNA specimens and their subsequent copy-number profiling at up to 50-kb resolution. Optimal use of these data for robust reconstruction of cancer cell phylogenies is a challenging computational problem calling for new and robust informatic and statistical tools.

In response, we used CORE to transform copy-number profiles of single cells into a form suitable for phylogeny. As a result of this transformation, each profile is characterized in terms of presence or absence of archetypal copy-number events recurrent in the single-cell populations. The entire set of copy-number profiles of single cells sampled from a tumor is summarized as an incidence table, essentially a matrix with cores as columns, single cells as rows, and elements quantifying, on a scale between 0 and 1, how well a given core is matched by a copy-number event in a given cell. Distances among cells as rows of this matrix can be readily computed and used for distance-based phylogeny. This methodology has been used to reconstruct the cell-population structure in a number of samples from prostate cancers. Importantly, this study shows that well-defined clones of tumor cells are likely to exist in prostates of patients with high, but not with low, Gleason scores. Furthermore, we see evidence that some of the clones spread to multiple anatomical sites within the affected prostate, including sites with less malignant histopathological characteristics.

Prediction of Response to Anthracycline Therapy in Breast Cancer

Anthracyclines are a class of chemotherapeutic drugs that are effective and widely used in treatment of a number of cancers, in particular those of breast. Sensitivity to these agents varies widely across the entire population of patients and likely depends on the pattern of genomic alterations in the tumor. Anthracycline administration also causes significant side effects, most importantly cardiotoxicity, and should therefore be restricted to patient populations for which the expected benefits outweigh these risks. Yet despite more than 50 years of use, the anthracycline mechanism of action is not firmly established, and there are as yet no reliable predictive markers. In response, we applied CORE to DNA copy-number profiles derived by the Hicks laboratory at CSHL from breast tumors of ~250 patients enrolled in the NEAT/BR9601 clinical trial in Britain. The study was conducted in collaboration with the Bartlett group at the Ontario Institute for Cancer Research. To discover markers of sensitivity to anthracyclines, we performed CORE analysis of the set of profiles and computed the incidence table. Next, we evaluated each core as a prospective marker of sensitivity and found dramatically higher benefit from treatment in patients positive for a narrow amplified region of chromosome 8. None of the marker-positive patients treated by anthracyclines have suffered a relapse in the 5 years since treatment, whereas the marker-positive patients who were not treated displayed a markedly higher rate of relapse than the remainder of the cohort. Given these results, a validation study has been initiated, with the goal to similarly analyze hundreds of breast tumor samples from the MA.5 clinical trial in Canada.

Significantly Distinct Branches of Hierarchical Trees

Hierarchical clustering (HC) is widely used as a method of partitioning data and of identifying meaningful data subsets. Most commonly, an application consists of visual examination of the dendrogram and intuitive identification of subtrees that appear clearly distinct from the rest of the tree. Obviously, results of such qualitative analysis and conclusions from it may be observer-dependent. Quantifying the interpretation of hierarchical trees and introducing mathematically and statistically well-defined criteria for distinctness of subtrees would therefore be highly beneficial.

We formulated and implemented a computational method termed tree branches evaluated statistically for tightness (TBEST) for identifying significantly distinct tree branches in hierarchical clusters. For each branch of the tree, a measure of distinctness, or tightness, is defined as a rational function of heights, both of the branch and of its parent. A statistical

procedure is then developed to determine the significance of the observed values of tightness. We tested TBEST as a tool for tree-based data partitioning by applying it to four benchmark data sets. These represent four distinct types of biological data: mRNA expression, DNA copy-number variation, proteomics, and flow cytometry. In each of the four cases, there is a well-defined and independently known partition of the data into classes. In all cases considered, TBEST reproduced these classes on par with or better than the existing techniques. The R language implementation of the method is now available, as an eponymous package, from the Comprehensive R Archive Network (CRAN).

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COMPUTATIONAL GENETICS

D. Levy

The data-rich environment at CSHL generates a wealth of opportunities for the application of mathematics and computation to further our understanding of biology and genetics. The primary activities of our lab are algorithm development, data analysis, and genetic theory, with a focus on sporadic human diseases such as autism, congenital heart disease, and cancer.

Autism Genetics and New Mutation

In collaboration with Michael Wigler and Ivan Iossifov, our efforts in autism attempt to bridge the gap between genetic theory and large-scale data analysis. In 2007, Zhao et al. (*Proc Natl Acad Sci 104:* 12831 [2007]) proposed a genetic theory of autism where new (or "de novo") mutations serve as an entry point for highly deleterious genetic lesions. This theory was supported by early studies showing an excess of de novo copy-number variations (CNVs) in autistic children and suggested a strategy for uncovering the set of genes whose disruption may result in autism. The Simons Simplex Collection (SSC) includes genetic samples and phenotypic data from families with exactly one child affected with an autism spectrum disorder (ASD). All 3000 families have samples collected from the mother, the father, the affected child, and typically at least one unaffected sibling. By identifying mutations that are present in the autistic child but absent in the parents and comparing to the observed rate in the unaffected siblings, we can infer a contributory rate for new mutations and identify likely genetic targets.

From 2008 to 2011, we generated and analyzed thousands of high-resolution CGH (comparative genome hybridization) arrays with 2.1 million data points per individual and, in the process, developed a set of tools to reduce system noise and uncover rare and de novo CNVs. The resulting study, published in 2011, included 1000 SSC families and established a rate of de novo CNVs of 8% in autistic children compared to 2% in their unaffected siblings. De novo CNVs were more frequent and larger in affected girls than affected boys, and we found evidence for excess transmission of rare variants to the affected children. In collaboration with Dorothy Warburton at Columbia University Medical Center, we conducted a similar study of 223 children with congenital heart disease (CHD) and found strong evidence of a causal role for de novo CNVs, with 10% of affected children having a de novo variant.

Although copy-number variants provide an excellent signal to background (4:1), even with the high resolution of the microarrays and the sophisticated informatics for denoising and analysis, most observable CNVs disrupt more than one gene. This necessarily complicates the effort to identify causal genetic targets. With the availability of low-cost targeted sequencing methods, the SSC data analysis effort switched to identifying de novo point mutations and small insertion or deletion events (indels) in the exome. Our results from the first 350 families, published in Iossifov et al. (*Neuron 74:* 285 [2012]), are in agreement with the results of the other groups studying exomic mutation: De novo "loss-of-function" variants, those that severely impair gene function, occur in 20% of affected children and in only 10% of their siblings. Together with groups at University of Washington and University of San Francisco, we are in the process of finishing the data analysis on the entire collection of SSC exomes. The latest data set identifies a strong signal for a causative role in missense mutations; however, the signal to background is small, on the order of one causal event against a background of 10 incidental ones.

Copy Number from Exome Sequence

Although we are no longer generating copy-number microarrays, we now have thousands of families with greater than 40× sequence coverage on the exome. The principal utility of these data is for its sequence content, but there is evidence that we can also extract copy-number variants. The primary challenge in identifying CNVs from exome capture data is that the capture process distorts coverage in ways that vary

from sample to sample. Our initial expectation was that capturing and sequencing the family together would result in the same systemic distortion for all members in the family. That proved not to be the case. Even samples captured together showed variability in their coverage distortion, likely resulting from differences in sample handling prior to applying the capture protocol (sample acquisition, DNA extraction, etc.). What is more, the entirety of the SSC exome project involves several different centers, each using their own protocols for capture and sequencing.

Fortunately, the variability expressed in an individual sample is often recapitulated on a population level, and thus, we can use our library of samples to identify the major elements of systemic noise. Our normalization procedure is coupled to a hidden Markov model (HMM) that integrates over all the copy-number possibilities for a given sample and returns a probability for each copy state for each region of the genome. We then modify the expected coverage based on the results of the HMM, repeat our normalization procedure, and iterate until the results are unchanged. After identifying likely regions of CNV, we apply statistical tests over the segmented events in each family to determine the plausibility of the CNV. These results include tests for goodness of fit, rarity of the event in the population, and its pattern of inheritance.

There are four objectives to our present copy-number study. The first is to increase the number of de novo copy-number variants associated with ASD. The second is to look for an imbalance in small de novo CNVs, events that were too small to detect using CGH technology but might be within our reach using the sequence data. The third is to test the claims with respect to rare inherited copy-number events and their unequal distribution to autistic children. Finally, we would like to combine the CNV analysis in the larger context of sequence data, for example, identifying SNPs that occur opposite a deletion and establishing parent of origin for de novo copy-number changes. The data analysis and algorithm implementations have been performed by Kith Pradhan and in collaboration with Chris Yoon, who has an alternative method for calling copy-number events.

From our current analysis, it appears that large de novo and transmitted events are easily identifiable in the exome data; however, finding small events continues to present a challenge. If our thresholds are too high, we fail to identify anything; if they are too low, we obtain many false events. Together with Zihua Wang in the Wigler lab, we are developing bench protocols that could test copy number at targeted positions in the genome using high-throughput sequencing methods. Such a protocol would provide an excellent screen for validating our calls on small events.

Insertions and Deletions by Exact Match

We are also developing, together with Peter Andrews at CSHL, an algorithm for identifying large genomic rearrangements—deletions, insertions, translocations, and inversions—by cataloging discontinuities in the mapping of reads against the reference genome. The majority of existing methods for finding such events approach the problem one read at a time, typically identifying a target region by anchoring a mate-pair and then fitting the misaligned read using a scoring algorithm with a mismatch/gap penalty. Elaborations on this method make a secondary correction to obtain consensus among gapped mappings per sample. These methods are reasonably good—provided the event is small and occurs in an uncomplicated region.

Our algorithm uses all the reads over the whole population to make inferences about genomic rearrangements. Thanks to Moore's law, computational methods that were impractical at the dawn of genomic analysis are now feasible, fast, and useful. Suffix arrays require vast amounts of memory but perform very fast lookups for a query sequence of all its unique maximal exact matches (MEMs) to the human genome. By cataloging and indexing all reads in a sample by their MEMs, in particular those with two distinct matches into the reference genome, we identify and label recurrently observed discontinuities. Integrating this information across all samples, we can recognize common events, spurious rearrangements resulting from sequence homology, and global discrepancies within the reference. With all reads indexed and anchored to the reference genome, we reassemble rare and de novo discontinuities within an individual or family. Early versions of this method using fixed-width exact matching and applied to the SSC exome data successfully identified large deletions, inversions, and insertions, and small indels, as well as rare and de novo pseudogenes. Our latest efforts show that the statistical signal is easily discernible even with just one distinct match into the reference genome, which should facilitate the identification of events that involve regions with multiple mappings in the genome.

Genetic Models of Autism

Autism is often described as the most highly heritable neurological disorder. Concordance for autism among identical twins is as high as 90%. There are several other interesting statistics in the epidemiology of autism that are less often noted. If a family has a single child with autism, the risk to the next born male child is ~20%. This elevated risk has led to an explanation that autism is a multifactorial disorder and that several gene disruptions are needed to produce an autism phenotype. That claim, however, is belied by another statistic: Given two children in the family with autism, the risk to a third-born male child is 50%.

One model that fits these data is to postulate two risk classes for autism: Nearly all families have a very low risk of having a child with autism, whereas a small percentage of families have a very high risk. Together with Michael Wigler and Swagatam Mukhopadhyay, we have been exploring simple genetic models that recapitulate the observed risk statistics, both for boys and girls, as well as observations that stem from genetic analyses of ASD families. Our model includes parameters such as de novo mutation rates, variability of penetrance, assortative mating, and the number and type of genetic targets.

Combinatorics allow for fast simulations of infinite populations, providing a means for quickly exploring the space of possible models consistent with the observable measures. We find that a simple model with two gene classes and different selection profiles for males and females is sufficient to match observable risk and mutation rates. This model also makes predictions about gender ratios as a function of severity, the role of transmitted mutation, and the genetic load in the population.

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COMPUTATIONAL SEQUENCE ANALYSIS

M. Schatz T. Garvin, A. Gupta, R. Gupta, J. Gurtowski, H. Lee, S. Marcus, G. Narzisi, D. Nettem, S. Ramakrishnan, A.H. Wences, G. Vurture

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 sequence data into complete genomes, for aligning sequences to discover variations or measure transcription levels, and for 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. Tyler Garvin is a new student from the Watson School of Biological Sciences. Tyler completed his undergraduate education in Biomedical Engineering at the University of Southern California. Throughout his dissertation, Tyler will be researching novel methods for single-cell DNA and RNA analysis with applications to cancer and other complex diseases. Srividya Ramakrishnan joined the lab as a Computational Scientific Analyst after completing her Master's degree in Bioinformatics from the New Jersey Institute of Technology. Srividya is working on building ultra-large-scale sequence analysis pipelines for KBase, the Department of Energy Systems Biology Knowledgebase. During the summer, Greg Vurture joined the lab through the undergraduate research program to develop new computational and mathematical approaches to infer the properties of genomes from unassembled sequencing reads. Avijit Gupta, Rushil Gupta, and Deepak Nettem completed their Master's degrees in Computer Science and have gone on to accept software engineering positions at Microsoft and TuneIn.

In the past year, our lab continued working on several funded projects studying the genetics of autism and of several important agricultural- and bioenergy-related plant species and developing algorithms for genome assembly and large-scale sequence analysis. In the last year, I have been interviewed by editors of the journals *Nature, Nature Biotechnology*, and *Nature Methods* as well as *Wired* Magazine and *The New York Times*, especially to describe my work applying third-generation sequencing technology from Pacific Biosciences to human and plant genetics.

Assemblathon 2: Evaluating De Novo Methods of Genome Assembly in Three Vertebrate Species

The process of generating raw genome sequence data continues to become cheaper, faster, and more accurate. However, assembly of such data into high-quality, finished genome sequences remains challenging. Many genome assembly tools are available, but they differ greatly in terms of their performance (speed, scalability, hardware requirements, acceptance of newer read technologies) and in their final output (composition of assembled sequence). More importantly, it remains largely unclear how to best assess the quality of assembled genome sequences. The Assemblathon competitions are intended to assess current state-of-the-art methods in genome assembly.

In Assemblathon 2, sequence data were provided for three vertebrate species (a bird, a fish, and a snake). This resulted in a total of 43 submitted assemblies from 21 participating teams. The assemblies were evaluated using a combination of optical map data, Fosmid sequences, and several statistical methods. From more than 100 different metrics, we chose 10 key measures by which to assess the overall quality of the assemblies. Our assembly of the fish genome placed second in the overall contest, using a new method prototyped by a former undergraduate research program (URP) participant in my lab, Paul Baranay.

Genome of the Long-Living Sacred Lotus *Nelumbo nucifera* (Gaertn.)

Sacred lotus is a basal eudicot of agricultural, medicinal, cultural, and religious importance. It was domesticated in Asia ~7000 years ago and cultivated for its rhizomes and seeds as a food crop. It is particularly noted for its 1300-year seed longevity and exceptional water repellency, known as the lotus effect. The latter property is due to the nanoscopic closely packed protuberances of its self-cleaning leaf surface, which have been adapted for the manufacture of a self-cleaning industrial paint, Lotusan.

The genome of the China Antique variety of the sacred lotus was sequenced with Illumina and 454 technologies, at respective depths of 101× and 5.2×. We assembled these data and achieved a contig N50 of 38.8 kbp and a scaffold N50 of 3.4 Mbp, which covers 86.5% of the estimated 929 Mbp total genome size. The genome notably lacks the paleotriplication observed in other eudicots, but it does reveal a lineage-specific duplication. The genome has evidence of slow evolution, with a 30% slower nucleotide mutation rate than observed in grape. Comparisons of the available sequenced genomes suggest a minimum gene set for vascular plants of 4223 genes. Strikingly, the sacred lotus has 16 COG2132 multicopper oxidase family proteins with root-specific expression; these are involved in root meristem phosphate starvation, reflecting adaptation to limited nutrient availability in an aquatic environment. The slow nucleotide substitution rate makes the sacred lotus a better resource than the current standard, grape, for reconstructing the pan-eudicot genome and should therefore accelerate comparative analysis between eudicots and monocots.

Cultivation and Complete Genome Sequencing of *Gloeobacter kilaueensis* sp. nov. from a Lava Cave in Kīlauea Caldera, Hawai'i

The ancestor of *Gloeobacter violaceus* PCC 7421T is believed to have diverged from that of all known cyanobacteria before the evolution of thylakoid membranes and plant plastids. The long and largely independent evolutionary history of *G. violaceus* presents an organism retaining ancestral features of early oxygenic photoautotrophs, and in whom cyanobacteria evolution can be investigated. No other *Gloeobacter* species has been described since the genus was established in 1974. *Gloeobacter*-affiliated ribosomal gene sequences have been reported in environmental DNA libraries, but only the type strain's genome has been sequenced. However, we report here the cultivation of a new *Gloeobacter* species, *G. kilaueensis* JS1T, from an epilithic biofilm in a lava cave in Kīlauea Caldera, Hawai'i. The strain's genome was sequenced from an enriched culture resembling a low-complexity metagenomic sample, using 9-kb paired-end 454 pyrosequences and 400-bp paired-end Illumina reads. The JS1T and *G. violaceus* PCC 7421T genomes have little gene synteny despite sharing 2842 orthologous genes; a comparison of the genomes shows they do not belong to the same species. Our results support establishing a new species to accommodate JS1T, for which we propose the name *Gloeobacter kilaueensis* sp. nov. Strain JS1T has been deposited in the American Type Culture Collection (BAA-2537), the Scottish Marine Institute's Culture Collection of Algae and Protozoa (CCAP 1431/1), and the Belgian Coordinated Collections of Microorganisms (ULC0316). The *G. kilaueensis* holotype has been deposited in the Algal Collection of the U.S. National Herbarium (US# 217948). The JS1T genome sequence has been deposited in GenBank under accession number CP003587. The complete genome sequence of *G. kilaueensis* JS1T may further the understanding of cyanobacteria evolution and the shift from anoxygenic to oxygenic photosynthesis.

The Advantages of SMRT Sequencing

Of the current next-generation sequencing technologies, single-molecule real-time (SMRT) sequencing from Pacific Biosciences is sometimes overlooked. However, attributes such as long reads, modified base detection, and high accuracy make SMRT a useful technology and an ideal approach to the complete sequencing of small and large genomes. The advantages the platform provides relate to the possibility of sequencing small and large genomes, with long non-amplified single-molecule sequencing, and on the capability of detecting methylated nucleotides.

Aluminum Tolerance in Maize Is Associated with Higher MATE1 Gene Copy Number

Genome structure variation, including copy-number variation (CNV) and presence/absence variation, contributes greatly to maize genetic diversity; however, its effect on phenotypes remains largely unexplored. Here, we describe how CNV underlies a rare allele that contributes to maize aluminum (Al) tolerance. Al toxicity is the primary limitation for crop production on acid soils, which make up 50% of the world's potentially arable lands. In a recombinant inbred line mapping population, copy-number variation of the Al tolerance gene multidrug and toxic compound extrusion 1 (MATE1) is the basis for the quantitative trait locus of largest effect on phenotypic variation. This expansion in MATE1 copy number is associated with higher MATE1 expression, which in turn results in superior Al tolerance. The three MATE1 copies are identical and are part of a tandem triplication, which we were able to resolve using PacBio SMRT long-read sequencing. Only three maize inbred lines carrying the three-copy allele were identified from maize and teosinte diversity panels, indicating that CNV for MATE1 is a rare, and quite likely recent, event. These maize lines with higher MATE1 copy number are also Al-tolerant, have high MATE1 expression, and originate from regions of highly acidic soils. Our findings show a role for CNV in the adaptation of maize to acidic soils in the tropics and suggest that genome structural changes may be a rapid evolutionary response to new environments.

The DNA Data Deluge

In June 2000, a press conference was held in the White House to announce an extraordinary feat: the completion of a draft of the human genome. For the first time, researchers had read all three billion of the chemical "letters" that make up a human DNA molecule, which would allow geneticists to investigate how that chemical sequence codes for a human being. In his remarks, President Bill Clinton recalled the moment nearly 50 years prior when Francis Crick and James Watson first discovered the double-helix structure of DNA. "How far we have come since that day," Clinton said.

But the president's comment applies equally well to what has happened in the ensuing years. In little more than a decade, the cost of sequencing one human genome has dropped from hundreds of millions of dollars to just a few thousand dollars. Instead of taking years to sequence a single human genome, it now takes ~10 days to sequence a half dozen at a time using a high-capacity sequencing machine. Scientists have built rich catalogs of genomes from people around the world and have studied the genomes of individuals suffering from diseases; they are also making inventories of the genomes of microbes, plants, and animals. Sequencing is no longer something only wealthy companies and international consortia can afford to do. Now, thousands of benchtop sequencers sit in laboratories and hospitals across the globe. Discussed here is the state of the art in sequencing and analysis methods.

Sixty Years of Genome Biology

Sixty years after Watson and Crick published the double helix model of DNA's structure, 13 members of *Genome Biology*'s Editorial Board selected key advances in the field of genome biology subsequent to that discovery. My response follows.

The most significant development in genome biology since 25 April 1953 has been the rise of large-scale DNA sequencing, pioneered by Fred Sanger in 1977 and refined over the last several decades in first-, second-, and now third-generation automated sequencing technologies. Determining the structure of DNA in 1953 was incredibly significant for determining the molecular framework for so many aspects of biology, including the frameworks for replication, transcription, inheritance, mutation and evolution, to name but a few. However, determining the structure of DNA is analogous to determining the structure of a piece of paper void of any literature or mathematics written upon it. Now, with the rise of high-throughput sequencing, we have the capability of reading the genetic messages written on those molecular pages. This capability has unlocked not only the human genome, but also those of thousands of other species, revealing their genes, regulatory sequences, and overall structures, which has in turn led to many important advances in biology and medicine. The next frontier in genome biology is to apply these technologies, and

the related molecular assays empowered by DNA sequencing, over large populations of species, individuals, and cells to compare, model, and predict how these systems behave.

The DNA60IFX Contest

In honor of the 60th anniversary of the publication of the structure of DNA, we organized a contest related to DNA and its applications in current research. The contest began on April 20 and ended on April 25, the anniversary itself, and popularly known as "DNA Day." The contest drew nearly 1000 participants from across the world. Reflecting the transition from genetics to genomics in the 60 years since the discovery, the contest was presented as a series of bioinformatics challenges in which participants would assemble, align, or otherwise analyze nucleic acid sequences to identify a message hidden in the data.

The contest consisted of five stages, ordered so that the solution to one stage unlocked access to the next by completing its URL. There were no timing requirements for the first four stages since they were released at a predefined time for all participants, although the overall winners were determined by how quickly they could correctly solve the final stage. The top prize was an iPad, and the second and third place entries had their choice of a one-year subscription to *Genome Biology* or registration to the Beyond the Genome conference. In addition to celebrating the discovery, we hoped to reach out to students and postdoctoral researchers around the world to motivate them to learn a few new techniques and a few new concepts of molecular biology. This appears to have been quite successful, and several students outside of biology participated in the contest. We hope and anticipate holding similar contests in the years to come.

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QUANTITATIVE BIOLOGY FELLOW

Quantitative Biology Fellows are independent researchers who enter this position soon after receiving a PhD. They come from the fields of mathematics, physics, engineering, or computer science and spend 3–5 years at CSHL applying techniques from these disciplines to important questions in biology.

Justin Kinney completed his PhD in Physics at Princeton University in 2008 and began his term as a Quantitative Biology Fellow in 2010. His research focuses on developing next-generation DNA sequencing as a tool for dissecting the structure and function of large macromolecular complexes. Of particular interest to his lab is the biophysical basis of transcriptional regulation—how simple interactions between proteins and DNA allow promoters and enhancers to modulate genes in response to physiological signals.

In 2010, Kinney and colleagues published a paper demonstrating Sort-Seq, a novel sequencing-based method that can measure the functional activity of hundreds of thousands of slightly mutated versions of a specific DNA sequence of interest. Using a novel information-theoretic analysis of the resulting data, Kinney et al. were able to quantitatively measure, in living cells, the protein–DNA and protein–protein interactions controlling mRNA transcription at a chosen promoter.

Kinney continues to develop this approach using a combination of theory, computation, and experiment. From a biological standpoint, Sort-Seq allows researchers to investigate important but previously inaccessible biological systems. Kinney's lab is currently using Sort-Seq to address open problems in transcriptional regulation, DNA replication, and immunology. These experiments also present new challenges for the field of machine learning, and a substantial fraction of Kinney's efforts are devoted to addressing the theoretical and computational problems relevant to the analysis of Sort-Seq data.

Transcriptional Regulation, Biophysics, and Machine Learning

J. Kinney

My research combines theory, computation, and experiment in an effort to better understand sequence-function relationships in biology.

My experimental work focuses primarily on the biophysics of transcriptional regulation in the gut bacterium *Escherichia coli*. In graduate school and as a postdoctoral researcher, I developed an experimental method called Sort-Seq that allows the transcriptional activities of hundreds of thousands of different *E. coli* promoters to be measured in a single experiment. These data can be used to build quantitative biophysical models for how elementary protein–DNA and protein–protein interactions combine to regulate gene expression. I continue to use Sort-Seq to study transcriptional regulation in *E. coli*, but am also using similar techniques to study both DNA replication initiation and antibody–antigen interactions.

The analysis of Sort-Seq data also highlights interesting problems in statistics and machine learning that have yet to be fully addressed. Approximately half of my research effort is devoted to these theoretical and computational questions.

Probability Density Estimation Using Scale-Free Field Theories

Analyzing Sort-Seq data requires the accurate estimation of continuous probability distributions, also known as "probability densities." Motivated by this need, I developed a method called density estimation using field theory (DEFT) to rapidly and accurately estimate probability densities using mathematical methods from theoretical physics (Fig. 1) (Kinney 2013). Unlike other density estimation approaches, this method has essentially no free tunable parameters. In particular, the length scale characterizing the smoothness of the underlying distribution is not set by

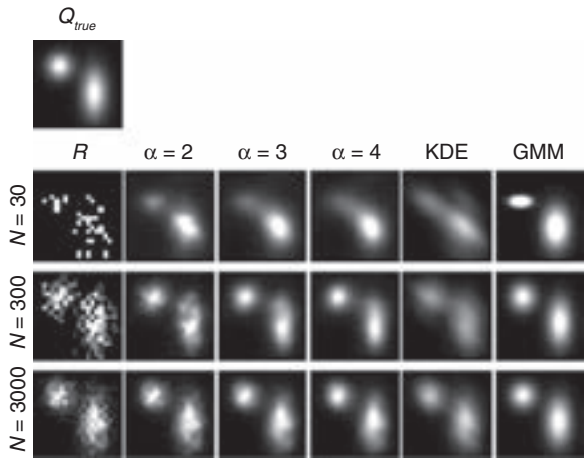

Figure 1. DEFT probability density estimation in two dimensions. Shown is a simulated probability density Q_{true} (composed of two Gaussian distributions), raw data sets R (of various size N) drawn from Q_{true}, and density estimates computed from R using either DEFT (with constrained derivative of order $\alpha = 2$, 3, or 4), kernel density estimation, or Gaussian mixture modeling.

the user, but is rather learned directly from the data themselves. My hope is that DEFT will be applicable to a wide range of statistical problems across many disciplines as well as to "Big Data" problems in industry.

Parametric Inference in the Large Data Limit Using Maximally Informative Models

Kinney and Atwal (2014a) describe a basic problem in statistical inference. Our work was initially motivated by my analysis of Sort-Seq data, but it is also relevant to the measurement of neuron receptive fields in sensory neuroscience.

Consider an experiment that produces an unlimited supply of data, for example, activity measurements for many different mutants of a bacterial promoter. In addition, assume, however, that the precise quantitative form of the experimental noise polluting these measurements is unknown. Such situations are common in Sort-Seq and other high-throughput biology experiments. However, not knowing how to model experimental noise causes problems for the standard statistical methods used to fit models to such data. The reason is that standard statistics requires that one compute "likelihood," and computing this requires an explicit quantitative model of experimental noise.

Atwal and I show that using a quantity from information theory called "mutual information" in place of likelihood circumvents the problem of not knowing the details of the experimental noise (Fig. 2). We also introduce the concept of "diffeomorphic modes"—directions in parameter space that mutual information cannot pin down. This work therefore reveals that different model parameters respond in qualitatively different ways to data. This is a general mathematical finding applicable to all statistical regression problems.

Equitability, Mutual Information, and the Maximal Information Coefficient

In Kinney and Atwal (2014b), we introduce a rigorous formulation of statistical "equitability," a heuristic criterion for measures of dependence that was recently advocated by Reshef et al. (*Science 334:* 1518 [2011]). We show that this formal criterion is a closely related core concept in information theory called the "Data Processing Inequality." We also show that this equitability criterion is naturally satisfied by the well-known mutual information measure and that estimates of mutual information made on finite data adhere well to this notion of equitability in practice.

Although the heuristic concept of statistical equitability is indeed valuable, we also find that the primary claims of Reshef et al. regarding equitability are incorrect. In particular, we show that the "maximal information coefficient" (MIC), a new statistic that Reshef et al. introduced in order to satisfy equitability, does not actually have this mathematical property. This finding is important because the paper by Reshef et al. has become one of the most highly cited statistics papers of the last few years, and MIC appears to have gained widespread use. We hope that our paper will motivate scientists in need of an equitable measure of dependence to use mutual information instead.

Ongoing Projects

A Biophysical Code for Transcriptional Regulation in *E. coli*

I have established a long-term collaboration with Rob Phillips (California Institute of Technology), the goal of which is to build a full biophysical model of the transcriptional regulatory code of *E. coli*. We are focusing on this bacterium for two reasons. First, the

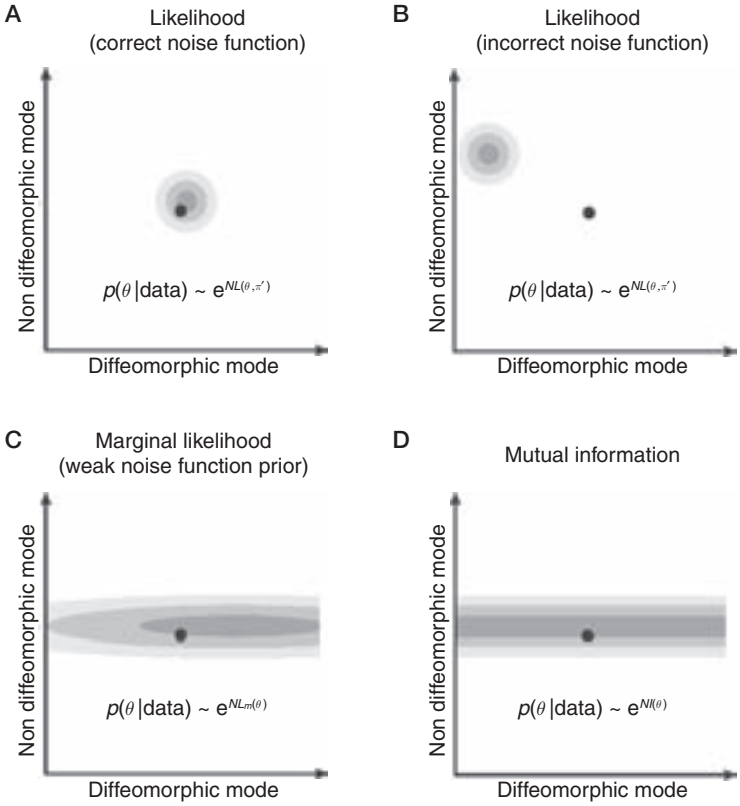

Figure 2. Schematic illustration of constraints placed on diffeomorphic and nondiffeomorphic modes in parameter space by different objective functions. The closed circle in each panel represents the correct model θ*; shades of gray represent the posterior distribution $p(\theta|\text{data})$. (*A*,*B*) Likelihood places tight constraints along both diffeomorphic and nondiffeomorphic directions in parameter space. (*A*) θ* will typically lie within error bars if the correct noise function π* is used to compute likelihood *L*. (*B*) However, if an incorrect noise function π′ is used, θ* will generally violate inferred constraints along both diffeomorphic and nondiffeomorphic modes. (*C*) Alternatively, if uncertainties in the noise functions are explicitly accounted for in the computation of $p(\theta|\text{data})$, one will find tight constraints on nondiffeomorphic modes but only weak constraints along diffeomorphic modes. (*D*) Mutual information places tight constraints on nondiffeomorphic modes but provides no constraints whatsoever on diffeomorphic modes. (Figure from Kinney and Atwal 2014a.)

underlying molecular biology of transcriptional regulation is understood well enough in *E. coli* to allow for mechanistic interpretations of Sort-Seq data. Second, transcription in *E. coli* is regulated in a hierarchical manner, with only a small number of proteins controlling a large number of promoters. Using in vitro single-molecule techniques together with in vivo Sort-Seq assays, we aim to systematically build a full sequence-dependent biophysical model for how regulatory proteins bind DNA, interact with one another, and modulate the rate of mRNA transcription. Initial work on this project has been pursued by Daniel Jones (California Institute of Technology), a graduate student who has spent his last four summers at CSHL doing experiments and computational work in my lab.

Sequence-Function Relationships in Antibody–Antigen Recognition

Despite the central role that antibodies have in the adaptive immune system and in biotechnology, surprisingly little is known about how antibody–antigen affinity depends on antibody sequence. For instance, how many possible antibodies can specifically bind an antigen of interest with 1 nM affinity, or with 1 pM affinity? Is the sequence-affinity landscape "convex" or "glassy"? Do the sequence-affinity landscapes for different antibody–antigen pairs exhibit any general features, for example, a universal "density of states." In collaboration with Aleksandra Walczak (ENS, Paris) and Thierry Mora (ENS, Paris), I am pursuing a combination of theory, computation, and experiment aimed at making progress on these important problems.

During the last year, Rhys Adams, a postdoctoral researcher working with Walczak, Mora, and myself, has been performing and analyzing Sort-Seq experiments designed to address these questions. Using yeast display technology, he is able to measure the binding affinities of thousands of mutant antibodies to a specific antigen of interest. Our hope is that these data will allow us to build quantitative models of the sequence-affinity landscape of this system, explore this landscape computationally, and look for features that might generalize to other antibody–antigen pairs.

The Biophysical Mechanisms of DNA Replication Initiation

I am collaborating with Bruce Stillman (CSHL) and Leemor Joshua-Tor (CSHL) on a project investigating the biophysical mechanisms of DNA replication initiation, both in vivo and in vitro. In collaboration with Bruce Stillman, I have developed a plasmid retention assay in the yeast *Saccharomyces cerevisiae* that produces Sort-Seq-like data probing the sequence dependence of DNA replication initiation. In parallel, the Joshua-Tor lab is performing in vitro binding assays of replication proteins to DNA replication origins. The primary goal of this project is to determine whether the in vitro sequence dependence of protein–DNA binding recapitulates the in vivo sequence dependence of replication initiation. My hope is that this approach will eventually produce insight into the biophysical mechanisms leading from protein–DNA binding to origin activation.

Formal Diagrammatic Methods for Biochemical Systems

All of my experimental projects require quantitative biophysical modeling of molecular interactions. However, the only systematic way to currently represent models of such interactions is to explicitly list all the possible states of a system, a task that becomes exponentially more difficult as system size increases. For this reason, I am developing a formal diagrammatic method for concisely and rigorously defining thermodynamic models of biochemical systems. This method allows equilibrium partition functions to be calculated systematically, and it should eventually facilitate nonequilibrium calculations as well. This approach can be applied to a wide range of processes in systems biology. My hope is that such methods will serve a purpose much like Feynman diagrams do in physics, bridging the gap between one's intuitive visual understanding of a system and rigorous quantitative models thereof.

PUBLICATIONS

Weirauch MT, Cote A, Norel R, Annala M, Zhao Y, Riley TR, Saez-Rodriguez J, Cokelaer T, Vedenko A, Talukder S, **DREAM5 Consortium**, et al. 2013. Evaluation of methods for modeling transcription factor sequence specificity. *Nat Biotechnol* **31:** 126–134.

In Press

Kinney JB, Atwal GS. 2014a. Parametric inference in the large data limit using maximally informative models. *Neural Comput* (in press).

Kinney JB, Atwal GS. 2014b. Equitability, mutual information, and the maximal information coefficient. *Proc Natl Acad Sci* (in press).

AUTHOR INDEX

WATSON SCHOOL OF BIOLOGICAL SCIENCES

ADMINISTRATION

Alexander Gann, PhD, WSBS Professor and Dean
Carrie Cowan, PhD, Associate Dean (from July)
Alyson Kass-Eisler, PhD, Postdoctoral Program Officer and Curriculum Director
Kimberly Creteur, MEd, MSEd, Admissions Coordinator
Kimberley Geer, Administrative Assistant

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Adrian R. Krainer
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David L. Spector (Director of Research)
David Stewart
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Professor, Department of Biology and Center for Cancer Research
Massachusetts Institute of Technology

WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

After a number of changes to the administrative staff at the WSBS last year, there was only one change in 2013. An Associate Dean, Dr. Carrie Cowan, joined the WSBS in July to help direct the long-term vision of the School. Carrie trained as a cell and developmental biologist at Wellesley College and University of California, Berkeley, where she obtained her PhD with Dr. Zac Cande. She performed postdoctoral work with Dr. Tony Hyman at the Max Planck Institute in Dresden, Germany, and then established an independent research group at the Research Institute of Molecular Pathology (IMP) in Vienna, Austria, studying cell polarity in *Caenorhabditis elegans* embryos. After mentoring several PhD and MS students in her lab and helping to restructure the PhD program in Vienna, Carrie comes to Cold Spring Harbor Laboratory to pursue her interest in graduate education and to help ensure that the Watson School continues to be a successful enterprise for both students and faculty.

NYS Accreditation

Carrie's first major project for the School was to prepare a lengthy self-study for the School's reaccreditation from the New York State Board of Regents. New York State conducted a site visit on October 31st and November 1st, meeting with senior laboratory administration, faculty, and students and exploring the Laboratory's facilities. Joining Department of Education representative Paul Thompson on the visit were biologists from other New York State institutions: James Boroweic (New York University), Ron Prywes (Columbia University), Wes Gruber (Columbia University), and Greg DeAngelis (University of Rochester). The site visitors will prepare a report of their findings that we should receive in early 2014. The Board of Regents will make the accreditation decision later in the year. We are confident that the visit went well and that accreditation will indeed be renewed.

The 10th WSBS Graduation

On April 28, 2013, we celebrated the Watson School's 10th graduation ceremony. Eight students were awarded PhD degrees: Eyal Gruntman from the Entering Class of 2006, Megan Bodnar, Joseph Calarco, Saya Ebbesen, Paloma Guzzardo, Maria Pineda, and Peter Znamenskiy from the Entering Class of 2007, and Felix Schlesinger from the Entering Class of 2008. Ralph Burgess from the Entering Class of 2007 was awarded a Master's degree, but he did not attend the ceremony. Honorary degrees were bestowed upon Jack Dixon and Brigid Hogan. Dr. Jack E. Dixon is a professor at the University of California, San Diego, and Vice President and Chief Scientific Officer of the Howard Hughes Medical Institute (HHMI). Since 2007, he has directed HHMI's flagship investigator program. Dr. Dixon was also responsible for identifying new opportunities that capitalize on the HHMI's expertise in biomedical research and science education. Dr. Brigid Hogan, who gave an inspirational commencement address, is the George Barth Geller Professor and Chair of the Department of Cell Biology, Duke University Medical Center. In 1983, she initiated the CSHL course "Molecular Embryology of the Mouse," one of the most influential courses in the Lab's history. Through this course and her lead role in writing and editing the CSHL Press manual that it spawned (*Manipulating the Mouse Embryo*, considered the "Bible" of mammalian embryo manipulation techniques), Dr. Hogan made the techniques of mammalian developmental genetics and experimental embryology widely available to the broader scientific community, greatly enhancing the work of others.

As with each graduation, we extended a special welcome to the family members and friends of our students who attend the ceremony.

2013 WSBS DOCTORAL RECIPIENTS

Student	Thesis advisor	Academic mentor	Current position
Megan Bodnar	David L. Spector	Nicholas Tonks	Postdoctoral Fellow, Mount Sinai School of Medicine (Advisor: Thomas Zwaka)
Joseph Calarco	Robert Martienssen	David Jackson	Postdoctoral Fellow, Stanford University (Advisor: Gerald Crabtree)
Saya Ebbesen	Scott Lowe	David Stewart	Postdoctoral Fellow, Memorial Sloan-Kettering Cancer Center (Advisor: Scott Lowe)
Paloma Guzzardo	Gregory Hannon	Adrian R. Krainer	Program Evaluation Consultant, Damon Runyon Cancer Research Foundation. Graduate Student Post, CSHL (Advisor: Gregory Hannon)
Marek Kudla	Gregory Hannon	David Jackson	Graduate Student Post, CSHL (Advisor: Gregory Hannon)
Hassana Oyibo	Anthony Zador	Hiro Furukawa	Graduate Student Post, CSHL (Advisor: Anthony Zador)
Michael Pautler	David Jackson	Robert Lucito	Research Associate, Trait Development, Vineland Research and Innovation Centre, Canada
Eugene Plavskin	Marja Timmermans	Jan A. Witkowski	Postdoctoral Fellow, New York University (Advisor: Mark Siegal)
Joshua Sanders	Adam Kepecs	Bruce Stillman	Graduate Student Post, CSHL (Advisor: Adam Kepecs)
Felix Schlesinger	Thomas Gingeras	Gregory Hannon	Bioinformatic Scientist, Illumina, Inc. San Diego, CA
Petr Znamenskiy	Anthony Zador	Terri Grodzicker	Postdoctoral Fellow, Biozentrum, University of Basel, Switzerland (Advisor: Thomas Mrsic-Flogel)

2013 WSBS graduates: (*Left to right*) CSHL President Bruce Stillman, Paloma Guzzardo, Petr Znamenskiy, Megan Bodnar, Saya Ebbesen, Maria Pineda, Joseph Calarco, Felix Schlesinger, WSBS Dean Alexander Gann

2013 THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2007

Megan Bodnar, March 29, 2013

The dynamics of pluripotency genes upon differentiation of mouse embryonic stem cells.

Thesis Examining Committee

Chair:	**Alea A. Mills**
Research Mentor:	**David L. Spector**
Academic Mentor:	**Nicholas Tonks**
Committee Member:	**Bruce Stillman**
External Examiner:	**Jane Skok,** *New York University*

Joseph Calarco, January 18, 2013

A system to study chromatin dynamics through pollen development.

Thesis Examining Committee

Chair:	**Gregory Hannon**
Research Mentor:	**Robert Martienssen**
Academic Mentor:	**David Jackson**
Committee Member:	**Leemor Joshua-Tor**
Committee Member:	**Adrian R. Krainer**
Committee Member:	**Danesh Moazed,** *Harvard University*
External Examiner:	**Craig Pikaard,** *Indiana University*

Saya Ebbesen, March 6, 2013

RNAi mouse models of breast cancer tumor suppressor genes.

Thesis Examining Committee

Chair:	**Lloyd Trotman**
Research Mentor:	**Scott Lowe**
Academic Mentor:	**David J. Stewart**
Committee Member:	**Gregory Hannon**
Committee Member:	**Scott Powers**
External Examiner:	**Ramon E. Parsons**, *Columbia University*

Paloma Guzzardo, February 12, 2013

Identification and characterization of novel components of the Drosophila *piRNA pathway.*

Thesis Examining Committee

Chair:	**Zachary Lippman**
Research Mentor:	**Gregory Hannon**
Academic Mentor:	**Adrian R. Krainer**
Committee Member:	**Leemor Joshua-Tor**
Committee Member:	**Emily Bernstein,** *Mount Sinai Hospital*
External Examiner:	**Victor Corces,** *Emory University*

Marek Kudla, July 23, 2013

Identification of miRNA target sites in mouse embryonic stem cells and derived cells of neuronal lineage.

Thesis Examining Committee

Chair:	**W. Richard McCombie**
Research Mentor:	**Gregory Hannon**
Academic Mentor:	**David Jackson**
Committee Member:	**Adrian R. Krainer**
Committee Member:	**Molly Hammell**
Committee Member:	**Marja Timmermans**
External Examiner:	**Ihor Lemischka,** *Mount Sinai Hospital*

Hassana Oyibo, June 3, 2013

A high-throughput sequencing approach to mapping synaptic connectivity in the brain.

Thesis Examining Committee

Chair:	**Pavel Osten**
Research Mentor:	**Anthony Zador**
Academic Mentor:	**Hiro Furukawa**
Committee Member:	**Gregory Hannon**
Committee Member:	**Josh Dubnau**
External Examiner:	**Lynn Enquist,** *Princeton University*

Michael Pautler, June 18, 2013

Meristem size and determinacy in maize: How stem cell activity shapes plant architecture.

Thesis Examining Committee

Chair:	**Robert Martienssen**
Research Mentor:	**David Jackson**
Academic Mentor:	**Zachary Lippman**
Committee Member:	**Hajime Sakai,** *University of Delaware*
External Examiner:	**Erik W. Vollbrecht**, *Iowa State University*

Eugene Plavskin, June 25, 2013

The story of an evolutionary hijacking: Regulation of the auxin response by an ancient small RNA pathway.

Thesis Examining Committee

Chair:	**Gregory Hannon**
Research Mentor:	**Marja Timmermans**
Academic Mentor:	**Jan A. Witkowski**
Committee Member:	**David Jackson**
Committee Member:	**Ralph Quatrano**, *Washington University, St. Louis*
External Examiner:	**Michael Scanlon,** *Cornell University*

Joshua Sanders, September 17, 2013

A framework for understanding decision confidence.

Thesis Examining Committee

Chair:	**Glenn Turner**
Research Mentor:	**Adam Kepecs**
Academic Mentor:	**Bruce Stillman**
Committee Member:	**Anthony Zador**
Committee Member:	**Carlos Brody**
External Examiner:	**Nathaniel Daw,** *New York University*

Petr Znamenskiy, March 19, 2013

Role of corticostriatal projections in auditory discrimination.

(*continued*)

2013 THESIS DISSERTATION DEFENSES (*continued*)

Thesis Examining Committee

Chair:	**Adam Kepecs**
Research Mentor:	**Anthony Zador**
Academic Mentor:	**Terri Grodzicker**
Committee Member:	**Anne Churchland**
Committee Member:	**Pavel Osten**
External Examiner:	**J. Anthony Movshon,** *New York University*

ENTERING CLASS OF 2008

Melanie Eckersley-Maslin, October 23, 2013

Characteristics of random monoallelic gene expression during embryonic stem cell differentiation.

Thesis Examining Committee

Chair:	**Thomas Gingeras**
Research Mentor:	**David L. Spector**
Academic Mentor:	**Gregory Hannon**
Committee Member:	**Robert Martienssen**
Committee Member:	**Christopher Vakoc**
External Examiner:	**Marisa Bartolomei,** *University of Pennsylvania*

Katie Liberatore, December 3, 2013

Mechanisms of plant reproductive success: Gene dosage, stem cell maintenance, and the regulation of shoot architecture.

Thesis Examining Committee

Chair:	**David Jackson**
Research Mentor:	**Zachary Lippman**
Academic Mentor:	**Adrian R. Krainer**
Committee Member:	**Marja Timmermans**
Committee Member:	**Doreen Ware**
External Examiner:	**Rüdiger Simon,** *Heinrich Heine University, Düsseldorf, Germany*

Zinaida Perova, August 13, 2013

Synaptic changes in the medial prefrontal cortex in resilience and susceptibility to stress.

Thesis Examining Committee

Chair:	**Stephen Shea**
Research Mentor:	**Bo Li**
Academic Mentor:	**Linda Van Aelst**
Committee Member:	**Fritz Henn**
Committee Member:	**Z. Josh Huang**
Committee Member:	**Pavel Osten**
External Examiner:	**Ming-Hu Han,** *Mount Sinai School of Medicine*

Felix Schlesinger, January 14, 2013

Discovery and analysis of transcription at regulatory elements.

Thesis Examining Committee

Chair:	**Mickey Atwal**
Research Mentor:	**Thomas Gingeras**
Academic Mentor:	**Gregory Hannon**
Committee Member:	**Alexander Krasnitz**
Committee Member:	**David L. Spector**
External Examiner:	**Manolis Kellis,** *Massachusetts Institute of Technology*

Teaching Award

At the graduation ceremony this year, Mickey Atwal was presented with the eighth annual Winship Herr Faculty Teaching Award, named in honor of the School's founding dean. Mickey, the lead instructor in the Specialized Disciplines course in Quantitative Biology, 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. Here is some of what they had to say about Mickey:

Alexander Gann (*left*) and Mickey Atwal

"Mickey was responsible for teaching subject matter that most of the class had little to no experience in and did so in such a way that engaged the class."

"He had no problem deviating from his scheduled lesson to go into detail about any topic that our class was either confused about or took specific interest in."

"Most of all, Mickey made quantitative biology fun and accessible to novices while still challenging us."

DOCTORAL THESIS RESEARCH

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2008			
Mitchell Bekritsky *Starr Centennial Scholar*	W. Richard McCombie	Michael Wigler	High-throughput microsatellite genotyping.
Dario Bressan *Robert and Teresa Lindsay Fellow*	Z. Josh Huang	Gregory Hannon	A novel technology for the space-specific recovery of biological molecules.
Sang Geol Koh *George A. and Marjorie H. Anderson Fellow*	Glenn Turner	Anthony Zador	Attention-dependent information routing in the mouse auditory cortex.
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.
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*	Gregory Hannon	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 *Caenorhabditis 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 *Drosophila 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.
ENTERING CLASS OF 2010			
Arkarup Bandyopadhyay *Goldberg Lindsay Fellow*	Zachary Lippman	Florin Albeanu	Identity and intensity encoding of odors in rodents.
Colleen Carlston *John and Amy Phelan Student*	Hiro Furukawa	Christopher Hammell	Identification and characterization of noise-suppressor genes that act via microRNAs in *Caenorhabditis elegans* larval development.
Matthew Koh *George A. and Marjorie H. Anderson Fellow*	Bo Li	Florin Albeanu	Roles of olfactory bulb inhibitory microcircuits in shaping the temporal response properties of mitral cells.
Lisa Krug *NIH Predoctoral Trainee*	Stephen Shea	Josh Dubnau	Mechanisms of transposon regulation in the central nervous system.

(*continued*)

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
John Sheppard *Bristol Myers Squibb/NIH Predoctoral Trainee*	Josh Dubnau	Anne Churchland	Neural mechanisms of multisensory decision making.
Jack Walleshauser *Barbara McClintock/NIH Predoctoral Trainee*	Christopher Hammell	Leemor Joshua-Tor	Structural basis for TUT4 uridylation of the pre-let-7/lin28 complex.
ENTERING CLASS OF 2011			
Robert Aboukhalil *NIH Predoctoral Trainee* *Starr Centennial Scholar*	Josh Dubnau	Mickey Atwal Michael Wigler	Using single-cell RNA-Seq to investigate tumor heterogeneity and evolution.
Brittany Cazakoff *Edward and Martha Gerry Fellow*	Christopher Hammell	Stephen Shea	Dynamic granule cell processing of odor information.
Joaquina Delas Vives *La Caixa Fellow*	Nicholas Tonks	Gregory Hannon	Functional role of long noncoding RNAs in hematopoiesis.
Anja Hohmann *David H. Koch Fellow*	John Inglis	Christopher Vakoc	Exploring the role of bromodomain-containing protein 9 (Brd9) in the maintenance of acute myeloid leukemia.
Justus Kebschull *David and Fanny Luke Fellow* *Genentech Foundation Fellow*	Marja Timmermans	Anthony Zador	Grasping the brain.
Fred Marbach *Farish-Gerry Fellow*	Josh Dubnau	Anthony Zador	A study of auditory corticostriatal cells in the behaving mouse.
Onyekachi Odoemene *NIH Predoctoral Trainee* *William Randolph Hearst Scholar*	Stephen Shea	Anne Churchland	The role of neural inhibition in perceptual decision making.
Sophie Thomain *George A. and Marjorie H. Anderson Fellow*	Josh Dubnau	Zachary Lippman	Characterization of a new meristem maintenance pathway in tomato and *Arabidopsis thaliana* and its relation to pollen tube growth.
Charles Underwood *William R. Miller Fellow*	Michael Schatz	Robert Martienssen	Epigenetic inheritance through mitosis and meiosis in *Arabidopsis thaliana*.
ENTERING CLASS OF 2012			
William Donovan *NIH Predoctoral Trainee*	Christopher Hammell	Josh Dubnau/ Anthony Zador	Transposons on the mind: Characterizing transposable element activity in TDP-43-pathology neurodegenerative disorders.
Talitha Forcier *NIH Predoctoral Trainee* *William Randolph Hearst Scholar*	Nicholas Tonks	Michael Wigler	Determining the chance of transcriptional events from single-cell RNA analysis.
Tyler Garvin *NIH Predoctoral Trainee/Dr. John and Consuelo Phelan Student*	Zachary Lippman	Michael Schatz	Structural variants and gene networks underlying complex human disease.
Yu-Jui (Ray) Ho *David and Fanny Luke Fellow*	Michael Schatz	Molly Hammell	Methods development for low-input RNA-Seq analysis.
Paul Masset *Florence Luke Fellow*	Jan A. Witkowski	Adam Kepecs	Representations of decision confidence in the brain: From Bayes' rule to channel rhodopsin.
Annabel Romero Hernandez *Genentech Foundation Fellow*	Adrian R. Krainer	Hiro Furukawa	Molecular mechanisms of inhibition in NMDA receptors.
Abram Santana *William Randolph Hearst Scholar*	Lloyd Trotman	David Tuveson	Pancreatic tumor biology and therapy.

Admissions 2013

The School received 274 applications for the 2013/2014 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 2013 entering class comprised myself, Gregory Hannon (chair), Mickey Atwal, Anne Churchland, Adrian R. Krainer, Zachary Lippman, W. Richard McCombie, Stephen Shea, Nicholas Tonks, and Linda Van Aelst.

Entering Class of 2013

On August 26, 2013, the Watson School welcomed a new class of 10 students: Giorgia Battistoni, Lital Chartarifsky, Sanchari Ghosh, Michael Giangrasso, Michael Gutbrod, Daniel Kepple, Laura Maiorino, Maria Nattestad, Kim Palacios, and Georgi Yordanov.

ENTERING CLASS OF 2013

Giorgia Battistoni, University of Pisa; MS Awarded: University of Pisa (2013); Scuola Normale Superiore Scholarship Recipient (2008)
Academic Mentor: Christopher Hammell

Lital Chartarifsky, Hebrew University of Jerusalem; MS Awarded: Hebrew University of Jerusalem (2012); Rector Prize Academic Excellence Award for MSc Studies: Hebrew University (2012); Dean's List Awarded: Hebrew University (2009–2011)
Academic Mentor: John Inglis

Sanchari Ghosh, Presidency College; Master of Biotechnology Awarded: All India Institute of Medical Sciences (2008); Ranked Second: All India Institute of Medical Sciences, Master of Biotechnology; Master of Biotechnology Student Fellowship, Department of Biotechnology, Ministry of Science and Technology (2006–2008); National Eligibility Test for Junior Research Fellowship (JRF) and Lectureship, Council for Scientific and Industrial Research Fellowship Scheme (2008); DBT-JRF Program, National Level Biotechnology Entrance Test (BET), Department of Biotechnology, Ministry of Science and Technology (2008); Junior Research Fellowship and Lectureship, National Entrance Test, University Grant Commission Fellowship Scheme (2007)
Academic Mentor: Josh Dubnau

Michael Giangrasso, Tulane University; Tulane University Distinguished Scholar (2009–2013); David H. Ledbetter Undergraduate Scholar (2010–2013); Dean's List Awarded: Tulane University (2010–2013); CELT Research Grant for Cellular Electrophysiology (2012–2013); Tulane University Neuroscience Department's Summer Research Program (2012)
Academic Mentor: Bo Li

Michael Gutbrod, University of Illinois at Urbana-Champaign; Dean's List Awarded: University of Illinois at Urbana-Champaign; Research Intern, Baxter Healthcare, Biochemistry and Cell Biology Group (Summer 2008, 2009)
Academic Mentor: Florin Albeanu

Daniel Kepple, Syracuse University; Harvard University NSF REU Summer Fellowship (2012); Harvard University Leadership Alliance Summer Research Early Identification Program Fellowship (2011); Gilman Scholarship, U.S. Department of State, Bureau of Educational and Cultural Affairs (2012); Syracuse University Anthony Yeh Scholarship (2012); Syracuse University Remembrance Scholarship (2011); Syracuse University Crown-Wise Award (2011); Syracuse University Sharon and Peter Kissel Endowed Scholarship (2009–2012)
Academic Mentor: David Stewart

Laura Maiorino, Università Vita-Salute San Raffaele; MS Awarded: Università Vita-Salute San Raffaele (2011); Postgraduate Research Fellow, Leukocyte Biology Unit, DiBit, San Raffaele Scientific Institute (2011–2013)
Academic Mentor: Nicholas Tonks

Maria Nattestad, University of the Pacific; University of the Pacific Honors Program; University of the Pacific Regents Scholar; Phi Beta Kappa; Phi Kappa Phi; The Rockefeller University Summer Undergraduate Research Fellowship (2012); Thomas J. Long Scholarship for Excellence in General Education; Raymond College Alumni Essay Competition Outstanding Entry; Mortar Board National Senior Honor Society; Alpha Lambda Delta Freshman Honor Society
Academic Mentor: Michael Schatz

Kim Palacios, Universidad Nacional Autonoma de Mexico; Universidad Nacional Autonoma de Mexico Honorific Award (2010–2011); Universidad Nacional Autonoma de Mexico Honorific Award, High Ranking (2010)
Academic Mentor: Bruce Stillman

Georgi Yordanov, University of Liverpool; University of Liverpool Sir Henry Wade Deacon Scholarship (2009–2013)
Academic Mentor: David Tuveson

2013 Entering Class: (*Top row, left to right*) Kim Palacios, Lital Chartarifsky, Laura Maiorino, Sanchari Ghosh. (*Bottom row, left to right*) Georgi Yordanov, Daniel Kepple, Giorgia Battistoni, Maria Nattestad, Michael Giangrasso, Michael Gutbrod

Academic Mentoring

The Watson School takes great pride in the level of mentoring that it offers its students. One example is our two-tiered mentoring approach whereby each student receives an academic mentor *and* a research mentor. Because WSBS students select a research mentor only in June right before their qualifying exam, the academic mentor is critical for monitoring students—and offering advice—during the intensive coursework of the first term, during student rotations, and when identifying a suitable research mentor. The academic mentors continue to follow the students throughout their doctoral experience, often serving as important advocates. 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 the Academic Mentors for the Entering Class of 2013.

STUDENT	MENTOR	STUDENT	MENTOR
Giorgia Battistoni	Christopher Hammell	Daniel Kepple	David Stewart
Lital Charatifsky	John Inglis	Laura Maiorino	Nicholas Tonks
Sanchari Ghosh	Josh Dubnau	Maria Nattestad	Michael Schatz
Michael Giangrasso	Bo Li	Kim Palacios	Bruce Stillman
Michael Gutbrod	Florin Albeanu	Georgi Yordanov	David Tuveson

The Fall Term Curriculum

Our faculty continues to do an outstanding job developing and delivering the curriculum. We are extremely grateful for their considerable time and effort in maintaining the high-quality coursework. The Curriculum Development and Integration Committee (CDIC)—Adrian Krainer (Chair), David Jackson, Nicholas Tonks, and Glenn Turner—continues to oversee development of 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

This year, we introduced some minor changes with regard to campus recruitment visits. Instead of visiting individual college campuses, we primarily focused on targeted visits to graduate fairs and minority conferences. In addition to these visits, the WSBS generated new mailing lists for the distribution of information to top undergraduate science departments around the world. A new poster and trifold pamphlet were designed for this recruitment season. A Quick Response (QR) barcode was created and added to printed materials, which when scanned sends interested students directly to the School's website. Additionally, emails were sent to personalized contacts and to an electronic mailing list of more than 50,000 individuals who receive information from the Cold Spring Harbor Laboratory Press or who have attended Meetings & Courses and Banbury. We are grateful to these departments for sharing their contact lists.

2013 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date
Cold Spring Harbor Laboratory Open House	Cold Spring Harbor Laboratory	March 23
American Association of Cancer Research Annual Meeting	Washington, D.C.	April 6–10
Molloy College Visit and Information Session	Cold Spring Harbor Laboratory	April 12
Brookhaven National Laboratory Graduate School Fair and Undergraduate Research Program Symposium	Brookhaven, New York	August 7
UMDNJ–NJ Medical School, PHRI Visit and Information Session	Cold Spring Harbor Laboratory	August 12
Binghamton University Graduate School Fair	Binghamton, New York	October 1
Cornell University Graduate School Day	Ithaca, New York	October 2
Society for Advancement of Chicanos and Native Americans in Science (SACNAS) National Conference	San Antonio, Texas	October 3–5
Atlanta University Center Consortium Graduate and Professional School Fair	Atlanta, Georgia	October 3
Princeton University Graduate and Professional School Fair	Princeton, New Jersey	October 4
Stanford University Graduate School Fair	Palo Alto, California	October 14
University of California, Santa Cruz Graduate and Professional School Fair	Santa Cruz, California	October 16
University of California, Berkeley Graduate School Fair	Berkeley, California	October 16
University of Cambridge Information Session	Cambridge, England	October 17
Columbia University Information Session	New York	October 17
Big 10+ Graduate School Exposition (Purdue University)	West Lafayette, Indiana	October 20–21
American Society for Human Genetics Annual Meeting	Boston, Massachusetts	October 22–26
California Forum for Diversity in Graduate Education Graduate School Fair	Moraga, California	October 26
Colby/Bates/Bowdoin Graduate School Fair Tour	Maine	October 28–29
University of Massachusetts Five College Graduate and Professionals Schools Information Day	Amherst, Massachusetts	October 30
Rutgers University Graduate & Professional School Fair	Rutgers, New Jersey	October 30
Society for Neuroscience Annual Meeting Graduate School Fair	New Orleans, Louisiana	October 14–15
Annual Biomedical Research Conference for Minority Students	Nashville, Tennessee	November 12–16
American Society for Cell Biology Annual Meeting	New Orleans, Louisiana	December 14–18

Interinstitutional Academic Interactions

WSBS students account for approximately half of the total graduate students at CSHL. The other half is 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 at CSHL. Currently, we have visiting students from institutions in Germany, France, and Spain, to name a few. The Watson School provides a contact person for the students and maintains relationships with administrations from their home institutions. These students are fully integrated into the CSHL community and receive all of the necessary assistance as they navigate the complexities of performing doctoral research away from their home institutions. The following students, listed in the table below, joined us from SBU this year.

STUDENT	CSHL RESEARCH MENTOR	AFFILIATION AND PROGRAM
Brinda Alagesan	David Tuveson	Stony Brook, Molecular Genetics and Microbiology
Kung-Chi Chang	David L. Spector	Stony Brook, Molecular and Cellular Biology
Michael E. Klingener	Gholson Lyon	Stony Brook, Genetics
Jesse M. Levine	Z. Josh Huang	Stony Brook, Neuroscience
Tobiloba Oni	David Tuveson	Stony Brook, Molecular and Cellular Biology
Justin Snider	Darryl Pappin	Stony Brook, Molecular and Cellular Biology
Yali Xu	Christopher Vakoc	Stony Brook, Molecular and Cellular Biology

Graduate Student Symposium

Each year, the students participate in three Graduate Student Symposia held at the Laboratory's Genome Research Center in Woodbury: one in January, one in May, and one in October. Each Symposium consisted of five sessions, where all of the presenters (senior students) each 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. We are grateful to the two student chairs of the Symposium, Nitin Shirole (SBU) and Lisa Krug (WSBS), and to Kimberley Geer for providing administrative oversight.

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 the Laboratory during 2013:

Postdoctoral Fellows

Katharine Borges	Santiago Jaramillo	Mukesh Lodha	Stephanie Shaw
Andres Canela Rodriguez	Marie Javelle	Martine Mirrione	Frederic Van Ex
Nabanita Chatterjee	Mads Aaboe Jensen	Hysell Oviedo	Angelina Vaseva
Muhan Chen	Ted Karginov	Jae-Hyun Park	Lang Son Vi
Ertugrul Dalkic	Tim Kees	Jennifer Parla	Fang Yang
Bernard Fendler	Brian Kolterman	Shilpi Paul	Jianchao Yao
Yael Galon-Wolfenson	Tali Lavy	Hongtao Qin	Zhu Zhu
Justyna Janas	Darrin Lewis	Kentaro Sahashi	

Graduate Students

Joseph Calarco	Jong Jin Han	Deepak Nettem	Areekul Sodsiri
Lorenza Calcaterra	Kyle Honegger	Jessica Oberheim	Ruei-Ying Tzeng
Li-An Chu	Canan Kuscu	Michael Pautler	Jie Wu
Laura Desban	Wangzhi Li	Megha Rajaram	Bin Xu
Saya Ebbesen	Willey Liao	Vincent Robert	Petr Znamenskiy
Eyal Gruntman	Elena Lum	Frederick Rollins	Giulia Zunino
Rushil Gupta	Antoine Molaro	Felix Schlesinger	

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 wish to thank faculty members Mickey Atwal, Mikala Egeblad, Adrian Krainer, David Spector, David Stewart, Linda Van Aelst, and Bo Li. Bo joined the committee in October, replacing Linda Van Aelst, who had completed her second consecutive 3-year term. Ioana Rus served until October, when she was replaced by Mathangi Ramesh. Colleen Carlston was the WSBS representative. We are thankful to Ioana, Mathangi, and Colleen, who contributed to discussions and provided useful suggestions and feedback.

Special Events: The Gavin Borden Fellow

The annual Gavin Borden Visiting Fellow (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 aimed primarily at the graduate students at the laboratory. Dr. Sean Carroll, a professor of molecular biology, genetics, and medical genetics at the University of Wisconsin, Madison, was this year's Gavin Borden Fellow. He studies the evolution of *cis*-regulation in the context of biological development, using *Drosophila* as a model system. Since 2010, he has also been vice president for science education of the Howard Hughes Medical Institute. His lecture, "Brave genius: A scientist's journey from the French resistance to the Nobel Prize," described the role of chance and circumstance in the life of the French scientist Jacques Monod. In addition, the students joined Dr. Carroll during a roundtable lunch and discussion at which he shared insights into his personal scientific journey with a discussion on "Science, education, and storytelling."

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 2013 programs possible, including the Annenberg Foundation, Lita Annenberg Hazen Foundation, Bristol-Myers Squibb Corporation, Mr. and Mrs. Richard M. Cashin, Mr. and Mrs. Landon Clay, Lester Crown, the Dana Foundation, Henriette and Norris Darrell, the Samuel Freeman Charitable Trust, the William Stamps Farish Fund, the Genentech Foundation, Mr. and Mrs. Alan Goldberg, Florence Gould Foundation, Gonzalo Río Arronte Foundation, William Randolph Hearst Foundation, Dr. Mark Hoffman, Annette Kade Charitable Trust, Mr. David H. Koch, Mr. and Mrs. Robert D. Lindsay and Family, Mr. and Mrs. David Luke III, Marjorie A. Matheson, 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, the Starr Foundation, and the Roy J. Zuckerberg Family Foundation.

We are also very fortunate to hold a prestigious National Research Service Award Predoctoral Training Grant from the National Institutes of Health, National Institute of General Medical Sciences. The School successfully competed for renewal of this award in 2013.

Student Achievements

The WSBS students continue to impress us all with their accomplishments. They publish their research findings in prestigious international journals—more than 240 to date—and obtain fellowships to pursue their research interests. In addition, our current students and alumni have been successful in receiving prestigious awards and fellowships. In 2013,

- WSBS student Brittany Cazakoff was awarded the 2012 Golden Key Graduate Scholar Award and a Graduate Scholarship from the Natural Sciences and Engineering Research Council of Canada (NSERC).

- WSBS graduates Ji-Joon Song and Zachary Lippman were promoted to Associate Professor.
- WSBS Student Melanie Eckersley-Maslin was awarded the poster prize at the EMBL meeting on Chromatin and Epigenetics, EMBO, Heidelberg.
- WSBS graduate Monica Dus received a National Institutes of Health K99/R00 Pathway to Independence Award.
- Three WSBS students, Joaquina Delas Vives, Anja Hohmann, and Justus Kebschull, were awarded predoctoral fellowships from Boehringer Ingelheim Fonds.

2013 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS

Aboukhalil R, Fendler B, Atwal G. 2013. Kerfuffle: A web tool for multi-species gene colocalization analysis. *BMC Bioinformatics* **14:** 22.

Batut P, Gingeras TR. 2013. RAMPAGE: Promoter activity profiling by paired-end sequencing of 5′-complete cDNAs. *Curr Protoc Mol Biol* **104:** 25B.11.1–25B.11.16.

Batut PJ, Dobin A, Plessy C, Carninci P, Gingeras TR. 2013. High-fidelity promoter profiling reveals widespread alternative promoter usage and transposon-driven developmental gene expression. *Genome Res* **23:** 169–180.

Bodnar MS, Spector DL. 2013. Chromatin meets its organizers. *Cell* **153:** 1187–1189. Review.

Campbell RAA, **Honegger KS**, Qin H, Li W, Demir E, Turner GC. 2013. Imaging a population code for odor identity in the *Drosophila* mushroom body. *J Neurosci* **33:** 10568–10581.

Castel SE, Martienssen RA. 2013. RNA interference in the nucleus: Roles for small RNAs in transcription, epigenetics and beyond. *Nat Rev Genet* **14:** 100–112. Review.

Das S, **Fregoso OI**, Krainer AR. 2013. A new path to oncogene-induced senescence: At the cross-roads of splicing and translation. *Cell Cycle* **12:** 1477–1479.

Dobin A, Davis CA, **Schlesinger F**, Drenkow J, Zaleski C, Jha S, **Batut P**, Chaisson M, Gingeras, TR. 2013. STAR: Ultrafast universal RNA-seq aligner. *Bioinformatics* **29:** 15–21.

Eckersley-Maslin MA, Bergmann JH, Lazar Z, Spector DL. 2013. Lamin A/C is expressed in pluripotent mouse embryonic stem cells. *Nucleus* **4:** 53–60.

Eveland AL, Goldshmidt A, **Pautler M**, Morohashi K, Liseron-Monfils C, Lewis MW, Kumari S, Hiraga S, Yang F, Unger-Wallace E, Olson A, Hake S, Vollbrecht E, Grotewold E, Ware D, Jackson D. 2013. Regulatory modules controlling maize inflorescence architecture. *Genome Res* **24:** 431–443.

Fregoso OI, Das S, Akerman M, Krainer AR. 2013. Splicing-factor oncoprotein SRSF1 stabilizes p53 via RPL5 and induces cellular senescence. *Mol Cell* **50:** 56–66.

Gruntman E, Turner GC. 2013. Integration of the olfactory code across dendritic claws of single mushroom body neurons. *Nat Neurosci* **16:** 1821–1829.

Guzzardo PM, Muerdter F, Hannon GJ. 2013. The piRNA pathway in flies: Highlights and future directions. *Curr Opin Genet Dev* **23:** 44–52. Review.

Hübner MR, **Eckersley-Maslin MA**, Spector DL. 2013. Chromatin organization and transcriptional regulation. *Curr Opin Genet Dev* **23:** 89–95.

Jiang K, **Liberatore KL**, Park SJ, Alvarez JP, Lippman ZB. 2013. Tomato yield heterosis is triggered by a dosage sensitivity of the florigen pathway that fine-tunes shoot architecture. *PLoS Genet* **9:** e1004043.

Li W, Prazak L, Chatterjee N, Grüninger S, **Krug L**, Theodorou D, Dubnau J. 2013. Activation of transposable elements during aging and neuronal decline in *Drosophila*. *Nat Neurosci* **16:** 529–531.

Lu X, Wontakal SN, Kavi H, Kim BJ, **Guzzardo PM**, Emelyanov AV, Xu N, Hannon GJ, Zavadil J, Fyodorov DV, Skoultchi AI. 2013. *Drosophila* H1 regulates the genetic activity of heterochromatin by recruitment of Su(var)3-9. *Science* **340:** 78–81.

Muerdter F, **Guzzardo PM**, Gillis J, Luo Y, Yu Y, Chen C, Fekete R, Hannon GJ. 2013. A genome-wide RNAi screen draws a genetic framework for transposon control and primary piRNA biogenesis in *Drosophila*. *Mol Cell* **50**: 736–748.

Nakasone ES, Askautrud HA, Egeblad M. 2013. Live imaging of drug responses in the tumor microenvironment in mouse models of breast cancer. *JVis Exp* **73:** e50088.

Pautler M, Tanaka W, Hirano HY, Jackson D. 2013. Grass meristems I: Shoot apical meristem maintenance, axillary meristem determinacy, and the floral transition. *Plant Cell Physiol* **54:** 302–312. Review.

Pi HJ, Hangya B, Kvitsiani D, **Sanders JI**, Huang ZJ, Kepecs A. 2013. Cortical interneurons that specialize in disinhibitory control. *Nature* **503:** 521–524.

Raddatz G, **Guzzardo PM**, Olova N, Fantappié MR, Rampp M, Schaefer M, Reik W, Hannon GJ, Lyko F. 2013. Dnmt2-dependent methylomes lack defined DNA methylation patterns. *Proc Natl Acad Sci* **110:** 8627–8631.

Sabin LR, **Delás MJ**, Hannon GJ. 2013. Dogma derailed: The many influences of RNA on the genome. *Mol Cell* **49:** 783–794. Review.

(*continued*)

2013 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS (*continued*)

Schlesinger F, Smith AD, Gingeras TR, Hannon GJ, Hodges E. 2013. De novo DNA demethylation and non-coding transcription define active intergenic regulatory elements. *Genome Res* **23:** 1601–1614.

Sheppard JP, Raposo D, Churchland AK. 2013. Dynamic weighting of multisensory stimuli shapes decision-making in rats and humans. *J Vis* **13**: 1–19.

Shi J, Whyte WA, **Zepeda-Mendoza CJ**, Milazzo JP, Shen C, Roe JS, Minder JL, Mercan F, Wang E, **Eckersley-Maslin MA**, et al. 2013. Role of SWI/SNF in acute leukemia maintenance and enhancer-mediated Myc regulation. *Genes Dev* **27:** 2648–2662.

Tanaka W, **Pautler M**, Jackson D, Hirano, HY. 2013. Grass meristems II: Inflorescence architecture, flower development and meristem fate. *Plant Cell Physiol* **54:** 313–324.

Tasdemir N, Lowe SW. 2013. Senescent cells spread the word: Non-cell autonomous propagation of cellular senescence. *EMBO J* **32:** 1975–1976. Review.

Vagin VV, Yu Y, Jankowska A, Luo Y, **Wasik KA**, **Malone CD**, Harrison E, Rosebrock A, Wakimoto BT, Fagegaltier D, Muerdter F, Hannon GJ. 2013. Minotaur is critical for primary piRNA biogenesis. *RNA* **19:** 1064–1077.

Vu TM, Nakamura M, **Calarco JP**, Susaki D, Lim PQ, Kinoshita T, Higashiyama T, Martienssen RA, Berger F. 2013. RNA-directed DNA methylation regulates parental genomic imprinting at several loci in *Arabidopsis. Development* **140:** 2953–2960.

Znamenskiy P, Zador AM. 2013. Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. *Nature* **497:** 482–485.

Watson School student

Alumni in Faculty and Senior Positions

To date, 66 students have received their PhD degree from the WSBS. Of these graduates, 15 currently hold tenure-track faculty positions and one holds an independent research position. Thirty-five of our former students are pursuing postdoctoral research, and 11 of our alumni completed their postdoctoral studies and have moved into positions in administration, education, industry, management consulting, and journal editing. Two are pursuing additional degrees.

WSBS GRADUATES IN FACULTY AND SENIOR POSITIONS

Name	Current position
François Bolduc	Assistant Professor, University of Alberta, Canada
Darren Burgess	Associate Editor, *Nature Reviews Cancer* and *Nature Reviews Genetics*, United Kingdom
Amy Caudy	Assistant Professor, University of Toronto, Canada
Daniel Chitwood	Assistant Professor, Donald Danforth Plant Science Center, St. Louis
Michelle Cilia	Research Molecular Biologist, U.S. Dept. of Agriculture, The Boyce Thompson Institute; Adjunct Assistant Professor, Cornell University
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	Assistant Dean, Florida State University
Zachary Lippman	Associate Professor, Cold Spring Harbor Laboratory
Marco Mangone	Assistant Professor, Arizona State University
Elizabeth Murchison	Reader, Cambridge University; Fellow, Kings College, United Kingdom

(*continued*)

WSBS GRADUATES IN FACULTY AND SENIOR POSITIONS (*continued*)

Name	Current position
Patrick Paddison	Assistant Member, Fred Hutchinson Cancer Research Center
Emiliano Rial-Verde	Associate Principal, McKinsey & Co., Inc., Geneva, Switzerland
Ji-Joon Song	Associate Professor, Korea Advanced Institute of Science and Technology (KAIST), South Korea
Niraj Tolia	Assistant Professor, School of Medicine, Washington University, St. Louis
Wei Wei	Assistant Professor, University of Chicago
Jeremy Wilusz	Assistant Professor, University of Pennsylvania (Feb. 2014)

Alexander Gann
WSBS Professor and Dean

SPRING CURRICULUM

Topics in Biology

ARRANGED BY **Alyson Kass-Eisler and Jan A. Witkowski**

FUNDED IN PART BY **The Daniel E. Koshland, Jr. Visiting Lectureship; the David Pall Visiting Lectureship; the Fairchild Martindale Visiting Lectureship; the Lucy and Mark Ptashne Visiting Lectureship; the Michel David-Weill Visiting Lectureship**

Each year, one or a team of invited instructors offer 7-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In Spring 2013 there were two such courses: Microbial Pathogenesis and Physical Biology of the Cell.

Microbial Pathogenesis

Attended by the entering classes of 2009 and 2012

INSTRUCTORS **Stanley Maloy,** Center for Microbial Sciences, San Diego
Ronald K. Taylor, Dartmouth Medical School

VISITING LECTURERS **Nicholas Biais,** CUNY Brooklyn College, New York
Lisa Craig, Simon Fraser University
Heran Darwin, New York University
Nancy Freitag, University of Illinois, Chicago

Throughout recorded history, microbial pathogens have been a major cause of human disease and mortality. However, with the advent of effective antibiotics, it seemed like the war on microbes had been won. Hence, for several decades, health-related research shifted to topics such as cancer, heart disease, and genetic diseases. Although research in microbial pathogenesis slowed, the microbes demonstrated the efficacy of evolution. Microbial resistance to antibiotics developed faster than new antibiotics could be made available, and the resistance spread throughout the microbial world. The global expansion of food distribution networks has increased the incidence of common microbial pathogens. Simultaneously, emerging microbial pathogens filled new ecological niches, such as indwelling medical devices that provide a surface for biofilms and the growing population of patients who are immunocompromised due to primary infections such as HIV or due to therapies for chronic diseases. Furthermore, recent discoveries have demonstrated that some diseases (e.g., ulcers) previously believed to be caused by a genetic predisposition or environmental conditions are actually caused by microbes. This microbial offensive

has summoned a renewed counterattack on microbial pathogens that has intensified during the last several years. Meanwhile, a variety of new tools have become available that make it possible to dissect the molecular basis of pathogenesis from both the microbial and host perspectives. Recently, the complete DNA sequence of bacterial pathogens has provided valuable insights into how microbial pathogens evolve and the extent of gene transfer between pathogens. These advances have revealed new ways to control infection, including the identification of novel targets for antimicrobials and novel approaches for vaccine development. Nevertheless, many more questions remain unanswered and many pathogens are still poorly understood.

Understanding bacterial pathogenesis demands a detailed knowledge of the host response as well as the pathogen itself. Both of these perspectives provide potential strategies for solving important clinical problems. To elucidate these distinct aspects of microbial pathogenesis requires an interdisciplinary approach, integrating the fields of microbiology, eukaryotic cell biology, immunology, and genomics.

This course focused on mechanisms of microbial pathogenesis and the host response, as well as the scientific approaches that are used to investigate these mechanisms. How do microbes adhere to host cells? How do environmental cues direct the response of microbial pathogens? How do microbial pathogens modulate host cells to expedite virulence? How do host cells respond to microbial pathogens? How does the host immune system react to microbial pathogens? What does genomics tells us about how microbial pathogens evolve? How do emerging pathogens take advantage of new ecological niches? Although there are numerous microbial pathogens, the answers to these questions indicate that many pathogens use similar approaches to solve common problems. The course integrated lectures by the instructors, directed readings of research papers, and seminars by the instructors plus three invited speakers who specialize in various aspects of bacterial pathogenesis. The course ran from Saturday, February 10, through Sunday, February 16.

Topics in Biology: Microbial Pathogenesis, 2013. (*Left to right*) Ronald Taylor, Lisa Craig, Nicolas Biais, Tyler Garvin, Silvia Fenoglio, Sho Goh, Nitin Chouhan, Ray Ho, Annabel Romero Hernandez, Will Donovan, Irene Liao, Paul Masset, Cinthya Zepeda Mendoza, Talitha Forcier, Charles Underwood, Ozlem Aksoy, Abram Santana, Kaja Wasik, Ian Peikon, Kristin Delevich, Stephane Castel, Sophie Thomain, Stanley Maloy

Physical Biology of the Cell

Attended by the entering classes of 2010 and 2011

INSTRUCTOR	**Rob Phillips,** California Institute of Technology
VISITING LECTURERS	**Joseph Blitzstein**, Harvard University **Eva-Maria S. Collins**, University of California, San Diego **Thomas Gregor**, Princeton University **Greg Huber**, University of California, Santa Barbara **Jané Kondev**, Brandeis University **Sanjoy Mahajan,** Franklin W. Olin College of Engineering
TEACHING FELLOWS	**James Boedicker**, California Institute of Technology **Franz Weinert,** 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. It began with "order of magnitude biology," showing how simple estimates can be exploited in biology. Simple models of a variety of different biological problems were constructed, 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 which are nearby in physical biology seem to be unrelated when viewed from the vantage point of molecular or cell biology. The instructors guided the students from start to finish on several modeling case studies. The course ran from Saturday, March 10, to Friday, March 16.

Topics in Biology: Physical Biology of the Cell 2013. (*Left to right*) Jané Kondev, Franz Weinert, Joe Blitzstein, Sanjoy Mahajan, James Boedicker, Joaquina Delas Vives, Robert Aboukhalil, Anja Hohmann, Fred Marbach, Kachi Odoemene, Justus Kebschull, Rob Phillips, Greg Huber, Colleen Carlston, John Sheppard, Brittany Cazakoff, Matt Koh, Arka Bandyopadhyay, Lisa Krug, Sophie Thomain, Jack Walleshauser

Teaching Experience at the DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Amanda McBrien (Lead)**
Jennifer Galasso
Elna Gottlieb
Erin McKechnie
Bruce Nash
Jermel Watkins

Science has an increasing role in society, and there is 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 experiences.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty 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, 18 WSBS faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Florin Albeanu
Mickey Atwal
Anne Churchland
Josh Dubnau
Mikala Egeblad
Hiro Furukawa
Christopher Hammell
Molly Hammell
Leemor Joshua-Tor
Adam Kepecs
Adrian R. Krainer
Zachary Lippman
Robert Martienssen
Michael Schatz
Stephen Shea
David Tuveson
Michael Wigler
Anthony Zador

FALL CURRICULUM

In the Fall of 2013, the major curriculum change was the introduction of a Molecular and Cell Biology "boot camp" at the start of the semester. The goal of this 3-day course was to introduce the students to the techniques and terminology of molecular biology that they will encounter in their subsequent courses. The boot camp featured six lectures from faculty members: Hiro Furukawa, W. Richard McCombie, David Spector, Lloyd Trotman, Christopher Vakoc, and Linda Van Aelst.

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

INSTRUCTORS	**Gregory Hannon (Lead)**	**Leemor Joshua-Tor**
	Linda Van Aelst (Co-lead)	**Bo Li**
	Alexander Gann	**Nicholas Tonks**
	Christopher Hammell	
GUEST LECTURERS	**Hiro Furukawa**	**Senthil Muthuswamy**
	Molly Hammell	**David Tuveson**
	Justin Kinney	**Christopher Vakoc**
	Robert Martienssen	
VISITING LECTURER	**Songhai Shi**, Memorial Sloan-Kettering Cancer Center	

A fundamental aspect of earning the PhD 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 include 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 spent an evening discussing the assigned articles with faculty. In a special module in the course, students participated in a mock study-section in which real National Institutes of Health R01 grants were reviewed and critiqued. This allows 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
Gene Regulatory Logic and the Construction of Multicellular Organisms: Insights from Flies, Plants, and Worms	Christopher Hammell
Signal Transduction and the Search for Novel Therapeutics	Nicholas Tonks
Study Section	Gregory Hannon Linda Van Aelst
The Brain: Wiring, Plasticity, and Maladaptation	Bo Li

The Darrell Core Course on Scientific Exposition and Ethics

INSTRUCTORS
David J. Stewart (Lead)
Mikala Egeblad
Arne Stenlund

GUEST LECTURERS
Lisa Bianco
Diane Esposito
Peter Tarr

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
Yaniv Erlich, The Whitehead Institute
Avner Hershlag, North Shore University Hospital
Amy Harmon, *The New York Times*
Boyana Konforti, Editor, *Nature Structural & Molecular Biology*
Ivan Oransky, Retraction Watch
Tung-Tien (Henry) Sun, New York University

The 2013 Scientific Exposition and Ethics (SEE) core course was led by David Stewart. He was joined by Arne Stenlund and a new addition to the course, Mikala Egeblad. 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 on society as well as the nature and boundaries of ethical behavior of scientists and their rights and responsibilities. A primary objective of the course was that students consider exposition and ethics 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.

Specialized Disciplines Courses

The students in the Entering Class of 2013 took a total of three Specialized Disciplines courses this fall: *Genetics and Genomics, Systems Neuroscience,* and *Quantitative Biology.* They will take the *Cellular Structure and Function* course at the start of 2014.

Genetics and Genomics

INSTRUCTORS **Thomas Gingeras (Lead)**
Zachary Lippman

GUEST LECTURERS **Josh Dubnau**
Marja Timmermans

This course placed modern genetics and genomics into the context of classical genetics. History, technique, and perspective of genetic inference were described around four levels of analysis: forward genetics, natural genetic variants, gene interaction, and genomics. Emphasis was on integrating classical with modern questions of genetic analysis: How are genes mapped and "cloned"? 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? What defines a gene and what gene variation exists in natural populations? What are the functional consequences of gene variation, and how is it detected? How are genomes organized and coordinately regulated? How can genomic information be catalogued, organized, and mined? These questions and concepts were fleshed out using examples from the literature.

Systems Neuroscience

INSTRUCTORS **Adam Kepecs (Lead)**
Glenn Turner

GUEST INSTRUCTORS **Anne Churchland**
Alexei Koulakov
Stephen Shea
Anthony Zador

This course introduced students to neuroscience, with a focus on learning and plasticity from its cellular basis, through development, to systems and behavior. Both experimental and theoretical viewpoints were explored. The course started with the basics of electrical signaling in neurons: ion channels, action potentials, and synaptic transmission. The cellular basis of learning, including Hebb's postulates, LTP (long-term synaptic potentiation), was discussed. The course explored the consequences of synaptic learning rules by examining how experience shapes the wiring of the nervous system during development and investigated how such building blocks translate into whole-organism behavior. The course then examined classical conditioning and asked how changes in synaptic transmission could underlie such behavior. Associative learning computational models of the learning process were discussed.

From behaviors that focus on simple memories, the course turned to behaviors that require making perceptual decisions. This was accomplished by covering some basic concepts of perceptual neuroscience, such as neuronal "receptive fields," and using these to discuss current results and models of perceptual decision making. Finally, the course turned to the learning of behaviors through reward and punishment, what is known as reinforcement learning. The course concluded with a discussion of the role of dopamine in reward and learning, the theory of reinforcement learning, and pathologies of reward-seeking behavior.

Quantitative Biology

INSTRUCTORS	**Mickey Atwal (Lead)** **Michael Schatz**
GUEST LECTURERS	**Ivan Iossifov** **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, the aim of this course was to equip the students with a basic training in modern statistical methods. By the end of the course, the students were able not only to answer many of the simple statistical questions that arise in data analyses, but also to become familiar with the more complex techniques used by fellow computational biologists. Topics covered included 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 introduced mathematical modeling motivated by the classic examples in quantitative biology such as the Delbrück-Luria experiment, Hopfield's kinetic proofreading, and Kimura's neutral theory of population genetics. For the first time, a 2-day Quantitative Biology boot camp, in which students were provided with the quantitative skills useful to their other coursework, was given at the very beginning of the fall semester.

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. Alyson Kass-Eisler, the Postdoctoral Program Officer, and Nicholas Tonks, the Scientific Director of the Postdoctoral Program, provide the day-to-day support for the postdoctoral program, which is overseen by the Dean of the Watson School of Biological Sciences (WSBS), Alexander Gann.

The Postdoctoral Liaison Committee (PDLC)—an elected group of postdocs who help share information and ideas between the administration and the postdoctoral community—continues to help enhance the postdoctoral experience at CSHL. The PDLC is the voice of the community and has regular meetings with CSHL President Bruce Stillman. In 2013, new member Saikat Nandi joined returning members Kate Creasey, Jonathan Ipsaro, Santiago Jaramillo, Dawid Nowak, and Benjamin Roche. The PDLC was been hard at work organizing two successful retreats this year aimed at fostering networking and collaboration. The day-long retreats took place at the Banbury Conference Center and included research talks, chalk talks, and discussions sessions. The PDLC also oversees and distributes funds to two career development groups as described below. These groups are primarily composed of postdoctoral fellows, but they also include graduate students. Today's postdocs face a number of challenges, including a very difficult job market. CSHL works hard to give its postdocs the upper hand when it comes to competing for the scarce jobs that are available. More and more, it has become our responsibility to introduce a diversity of career opportunities that are available and to provide the tools to our postdocs to prepare for these positions. Thanks to the assistance from PDLC and these career development groups, a number of fantastic events were organized.

The Career Development Program (CDP) is interested in providing programming geared toward careers in academia. They continued the successful "Conversations with Faculty" series in which the postdocs were given career insights in an informal and interpersonal format. Sessions included First Year as a P.I., led by CSHL Assistant Professors Michael Schatz and Anne Churchland, and Going on the Job Market: Applicant Perspective, led by two current postdocs Martine Mirrione, from Bo Li's laboratory, and Santiago Jaramillo, from Tony Zador's laboratory who were successful in their academic job search this year.

The CDP also hosted another extremely well-received workshop on preparing for the Chalk Talk, an integral part of the academic job search. In this session, Muhan Chen, a postdoctoral researcher in Lloyd Trotman's laboratory, presented her future research plans in front of Adrian Krainer, Alea Mills, and Hongwu Zheng, who served as the mock hiring panel.

Together, we have also been working on building connections with local colleges and universities to provide teaching experience opportunities for interested postdocs. The first of these

connections has been made with Molloy College. There are plans in place to expand this program in 2014. In addition, selected postdoctoral fellows participate in the Watson School as tutors either one-on-one or in the classroom setting, thus providing the postdocs with good experience in teaching and mentoring.

Finally, the CDP also has a series called "Getting to Know Your Faculty" where CSHL faculty members share stories of their journey throughout their career and highlight the person behind the scientific concepts and publications. The guests this year were Leemor Joshua-Tor and Michael Wigler.

The Bioscience Enterprise Club (BEC) is most interested in providing information for nonacademic careers, and they have also been organizing a series of seminars and workshops. The areas of interest include biotechnology, intellectual property, law, regulatory affairs, and venture capitalism. This year's guests included Jennifer Henry, Director of Life Sciences at the New York Academy of Sciences; Gustavo Stolovitzky, manager of the IBM System Biology Group; David Frendewey, Sr. Director from Regeneron Pharmaceuticals, Inc.; Jing Li, Senior Scientist, Merck & Co.; John H. Friedman, Founding and Managing Partner of Easton Capital Group; Daniel Nolan, Director of Research, Angiocrine; and Matthew Kaplan, Managing Director, Ladenbury Thalman & Co. Through BEC, CSHL postdocs have also been invited to networking events and lectures sponsored by Topspin Partners, Canrock Ventures, and Accelerate Long Island, three local organizations.

The postdoc and student leaders of BEC—Dawid Nowak, Assaf Vestin, and Robert Aboukhalil—successfully applied for funding from the American Society for Cell Biology to fund their inaugural "Beyond the Bench" symposium for alternative careers in academia. The Symposium consisted of talks, live Q&As, and panels about career opportunities for PhDs interested in careers in biotechnology, entrepreneurship, publishing, science writing, and IP/law. The keynote speakers were Dr. Herb Boyer, cofounder of Genentech and 1996 recipient of the Lemelson-MIT Prize, and Dr. Colin Goddard, Chairman & CEO of Coferon and former CEO of OSI Pharmaceuticals.

As a core component of the laboratory's National Cancer Institute–funded postdoctoral training grant, a course on the Fundamentals of Cancer was held at the Banbury Conference Center. The 3-day course was co-organized by Program Director and CSHL faculty member Alea Mills and Hannes Vogel from Stanford University. The course included lectures from CSHL faculty and a hands-on workshop for discussing case studies of actual cancer patients.

To celebrate National Postdoc Appreciation week, we held a faculty/postdoc volleyball game, barbeque, and events fair highlighting the career development initiatives. It was a great opportunity for the community to get together not only for some fun (particularly when the postdocs won the match), but also to network and find out about the ongoing programs we have been running.

Through the Laboratory's Human Resources department, the postdocs have had access to free jobseeker webinars hosted by the Higher Education Research Consortium. The 2013 webinars included Succeeding at the Academic Job Search in the Sciences, Academic Careers in America's Two-Year Colleges, How to Get Published, Publishing Ethics, Interviewing!, and Making Your Case: Writing Cover Letters for Nonacademic Jobs.

To make the most of their experience here, CSHL Director of Research David Spector has been hosting quarterly talks with recently arrived postdocs to provide them with an orientation highlighting the opportunities available to them at CSHL. It is also a nice opportunity to get to know the new postdocs and for them to meet one another.

Each year, during the Laboratory's annual In-House Symposium, a prize is awarded by the Watson School for the best poster by a postdoctoral fellow and the best poster by a graduate student. In addition to providing a forum for the postdoctoral fellows to show off their research, and potentially win a prize, the poster session gives the entire scientific community a chance to come

together and share ideas beyond the walls of their individual laboratories. It has been a great success for both the presenters and the Laboratory community as a whole. This year, the postdoctoral prize was shared between Dannielle Engle from David Tuveson's laboratory and Yichun Shuai from Yi Zhong's laboratory.

Demystifying Science, which started in 2011, enlists postdoctoral fellows who bring their expertise and teaching interests to the educationally diverse staff of the Laboratory. In the 2013 sessions, geared toward a general audience, postdocs have talked about the following: From CSHL to CVS: How Discoveries in the Lab May Turn into Drugs, by Jackie Novatt; Seeing Is Believing: Advancing Whole Brain Imaging, by Arun Narasimhan; Follow Your Nose: Social Memory Formation in Mice, by Dennis Eckmeier; Mind the Gap: Brain Cells under the Magnifying Glass, by Francesca Anselmi; The Social Brain: Autism and Brain Activity Maps, by Yongsoo Kim; and Understanding Cancer, One Cell at a Time, by Robert Aboukhalil.

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 Writing for Biomedical Publication; From Idea to IPO; The Nonacademic Job Search: Target the Job, Tailor the Approach; Introduction to Scientific Teaching; Risky Business: A Pharmaceutical Industry Strategy Workshop; PepsiCo Journey through Science Day; From Scientist to CSO: A Leadership Prep Course for Scientists in Business, Academia, Government, and Research Institutes; Powerful Teaching Statements for STEM Educators: An Interactive Workshop for Scientists and Teachers; and Scientists Teaching Science. In addition, the biannual symposium What Can You Be with a PhD? was held November 2–3 at New York University.

On November 14, 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; The Grant Abstract/Public Summary; Peer Review Panel Discussion; Effective Writing Skills; and Fellowships: Career Development Awards and Resources for Postdocs.

Postdoctoral fellows at CSHL compete successfully for awards and fellowships. Of note this year, Emily Hodges, a postdoctoral fellow in Gregory Hannon's laboratory, was a finalist for the prestigious Blavatnik Award from the NYAS. In addition, four postdocs—Sandra Ahrens from Bo Li's lab, Guy Horev from Alea Mills' lab, Yongsoo Kim from Pavel Osten's lab, and Keerthi Krishnan from Josh Huang's lab—received Young Investigator grants from the National Alliance for Research on Schizophrenia and Depression (NARSAD).

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 2013, the Laboratory's departing postdoctoral fellows went on to positions at Bayer CropScience, City College of New York, Oncotest (Germany), Hunan University (China), inGenious Targeting Laboratory, Merck & Co., Quinnipiac University, Santaris Pharma (Denmark), University of California at Riverside, and the University of Oregon, just to name a few.

UNDERGRADUATE RESEARCH PROGRAM

PROGRAM DIRECTORS **Anne Churchland**
Michael Schatz

PROGRAM ADMINISTRATOR **Kimberly Creteur**

Established more than 50 years ago, the CSHL Undergraduate Research Program (URP) plays a major role in providing and setting the standard for meaningful hands-on undergraduate research training in biology. The 10-week program begins the first week of June. During the first week, the students are oriented to the campus, receiving a guided historical tour of campus and a tour of all facilities and resources available to them; this ensures 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, and 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 2013 were a pizza party 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 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 2013 URP participants on Blackford Lawn.

The following 27 students, selected from 738 applicants, took part in the 2013 program:

Lauren Choate, Truman State University
Advisor: **Marja Timmermans**
Funding: **Dorcus Cumming Scholar**
Mapping and characterization of a leaf polarity mutant in maize: rld-5409.

Amy Danson, University of Cambridge
Advisor: **David Tuveson**
Funding: **Genetics Society of the United Kingdom**
Optimizing growth conditions of normal and diseased pancreatic organoids to study and identify pancreatic cancer biomarkers.

Abhishek Dev, Bard College
Advisor: **Adam Kepecs**
Funding: **Thirtieth Anniversary URP Scholar**
Effect of morphine on decision making.

Maria Eguiluz, Hope College
Advisor: **Gregory Hannon**
Funding: **Student selected by HHMI-EXROP**
Characterization of nuclear protein CG13741 in the germline piRNA pathway.

Michael Fishman, Swarthmore College
Advisor: **Pavel Osten**
Funding: **Alfred L. Goldberg Fellowship**
The role of the medial amygdala and ventromedial hypothalamus circuit in mouse social behavior.

Emily Flynn, Smith College
Advisor: **Thomas Gingeras**
Funding: **National Science Foundation Scholar**
Examining RNA annotation and quantification by RAMPAGEL comparison with RNA-Seq and Pol II.

Gregory Fuller, Johns Hopkins University
Advisor: **Z. Josh Huang**
Funding: **Burroughs Wellcome Fellowship**
Chandelier cells and apoptosis.

Heather Fuller, University College London
Advisor: **Josh Dubnau**
Funding: **Genetics Society of the United Kingdom**
Gypsy virus and neurodegenerative disorders.

Michael Jacobs, Oberlin College
Advisor: **Leemor Joshua-Tor**
Funding: **James D. Watson Fellow**
CG3893 and the piRNA pathway.

Victoria Jones, North Carolina Central University
Advisor: **Alea A. Mills**
Funding: **National Science Foundation Scholar**
The role of plant homeodomains (PHDs) of chromodomain helicase DNA-binding protein 5 in neural stem cells.

David Kleinman, University of Toronto
Advisor: **Lloyd Trotman**
Funding: **Robert H.P. Olney Fellow; Garfield Fellowship**
STAT3 inhibition and prostate cancer.

Ricki Korff, Cornell University
Advisor: **Mickey Atwal**
Funding: **National Science Foundation Scholar**
Germline genes and cancer.

Prashant Kota, Rensselaer Polytechnic Institute
Advisor: **Gholson Lyon**
Funding: **Howard Hughes Medical Institute Scholar**
Investigation of protein—protein interactions in the amino-terminal acetyltransferase complex.

Therese LaRue, Skidmore College
Advisor: **David Jackson**
Funding: **Former URP Fund Scholar**
Identifying direct targets of FEA4, a master regulator of meristem size in maize.

Yitong Li, Cornell University
Advisor: **Hiro Furukawa**
Funding: **Libby Fellowship**
New approach aided the study of human SPPL2b in oligomerization and protease activity.

Abigail Lin, Duke University
Advisor: **W. Richard McCombie**
Funding: **National Science Foundation Scholar**
Classifying epistasis in the DISC1 interactome.

Michael MacGillivray, University of Notre Dame
Advisor: **Alexander Krasnitz**
Funding: **University of Notre Dame URP Scholar**
Mathematical inference of tumor phylogeny.

Pascal Maguin, Hunter College, SUNY
Advisor: **Mikala Egeblad**
Funding: **Hunter College Scholar**
Exploration of LOXL2 expression in pancreatic cancer.

Uju Momah, Amherst College
Advisor: **Bruce Stillman**
Funding: **National Science Foundation Scholar**
Nucleosome disassembly ahead of the DNA replication fork: In vivo studies.

Marjorie Morales, Stony Brook University
Advisor: **Leemor Joshua-Tor**
Funding: **Student selected by HHMI-EXROP**
Argonautes and GW182 proteins in microRNA-mediated gene silencing.

Holly Rees, University of Cambridge
Advisor: **Adrian R. Krainer**
Funding: **James D. Watson Undergraduate Scholar**
Investigating the effect of SRSF1 on nonsense-mediated mRNA decay (NMD).

Benjamin Schuman, State University of New York at Geneseo
Advisor: **Stephen Shea**
Funding: **Von Stade Fellowship**
Locus coeruleus activity in response to social stimuli.

Daniel Starer-Stor, Oberlin College
Advisor: **Anthony Zador**
Funding: **William Townsend Porter Foundation Scholar**
Generation of random barcodes for in vivo cell identification.

Alexis Tchaconas, Columbia University
Advisor: **Michael Wigler**
Funding: **National Science Foundation Scholar**
Looking beyond the nucleus: Mitochondrial DNA transmission in autism spectrum disorder.

Akash Umakantha, Vanderbilt University
Advisor: **Partha Mitra**
Funding: **William Shakespeare Fellowship**
Addition of high-resolution Nissl histology to Waxholm space.

Gregory Vurture, New York University
Advisor: **Michael Schatz**
Funding: **National Science Foundation Scholar**
Mathematics of genome architecture.

Alissa Williams, Wofford College
Advisor: **Gregory Hannon**
Funding: **National Science Foundation Scholar**
Viral barcode tracking on clonal tumor formation.

PARTNERS FOR THE FUTURE

PROGRAM DIRECTOR **David Jackson**

PROGRAM ADMINISTRATOR **Michele Borriello**

The Partners for the Future Program, established by Dr. James Watson in 1990, provides an opportunity for talented Long Island high school students to have hands-on experience in biomedical research at Cold Spring Harbor Laboratory. This highly competitive program is open to Long Island high school students in their junior year. Each high school science chairperson may nominate up to three students. The top candidates are interviewed by CSHL scientists. Students selected to the program are paired with a scientist mentor and spend a minimum of 10 hours per week, September through March of their senior year, conducting original research. At the conclusion, the students present their projects to an enthusiastic audience of the students, scientific mentors and colleagues, CSHL administrators, parents, and teachers. Although the students learn a great deal about modern biology and state-of-the-art research techniques, the main advantage of the program is that they are exposed to day-to-day life 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 2013–2014 Partners for the Future were chosen from among 54 nominations. They are listed below.

Partner	High School	Laboratory	CSHL Mentor
Yohance Basdeo	Valley Stream South High School	Adrian R. Krainer	Tomoki Nomakuchi
Nathaniel Hogg	Friends Academy	Adam Kepecs	Hyun Jae Pi
Priyanka Kumar	Syosset High School	Mikala Egeblad	Mario Shields
Rebecca Layne	Oyster Bay High School	Robert Martienssen	Kate Creasey
Sarah Lee	Mineola High School	David Jackson	Qingyu Wu
Ann Lin	Farmingdale High School	Robert Martienssen	Rowan Herridge
Marissa Mathew	Commack High School	Doreen Ware	Lifang Zhang
Paige Varney	Hicksville Senior High School	Alea Mills	Alea Mills
Lindsey Stevens	East Hampton High School	David Tuveson	Vincenzo Corbo
Katherine Vera	Farmingdale High School	David Jackson	Huyen Bui
Dylan Wadler	Harborfields High School	Adrian R. Krainer	Isabel Aznarez Da Silva
George Wang	Syosset High School	Leemor Joshua-Tor	Ante Tocilj

Partners for the Future participants

MEETINGS & COURSES PROGRAM

ACADEMIC AFFAIRS

The Cold Spring Harbor Laboratory Meetings & 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. The Meetings & Courses Program at the Laboratory attracted strong attendance with more than 7300 meeting participants and almost 1300 course participants (trainees, teaching, and support faculty). The Cold Spring Harbor Asia Program, including 17 conferences and one summer school, has to date attracted more than 3000 participants, bringing the anticipated year-end total for both the United States- and China-based programs to almost 11,600.

The Laboratory held 27 academic meetings this year, which brought together scientists from around the world to discuss their latest research. The 78th Symposium in this historic series addressed Immunity and Tolerance and attracted almost 400 participants, including notable immunologists James Allison, Frederick Alt, David Baltimore, Michel Nussenzweig, Anne O'Garra, William Paul, Hidde Ploegh, Fiona Powrie, Lalita Ramakrishnan, Stephen Smale, Tadatsugu Taniguchi, Craig Thompson, and Irving Weissman.

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 will be presented and that young scientists will have the chance to describe their work. The introduction of several successful new meetings occurred in 2013 with Wiring the Brain, Metabolic Signaling, Disease: From Cell to Organism, and Behavior and Neurogenetics of Nonhuman Primates. The annual meetings on the Biology of Genomes and Retroviruses were again oversubscribed, and many others attracted strong attendance despite the funding climate. Many of these meetings have become essential for those in the field and are held on a biannual basis. Two special meetings on Genes and Diagnostics: A Myriad of Issues in Biotech IP and the History of Restriction Enzymes drew a rather different audience, respectively, of lawyers and historians. Partial support for individual meetings is provided by grants from the National Institutes of Health (NIH), the National Science Foundation (NSF), and other foundations and companies. Core support for the meetings program is provided by the Laboratory's Corporate Sponsor Program.

Symposium Wine and Cheese

The Courses program covers a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of the courses remains to teach advanced students cutting-edge science 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, lecturers, and assistants come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor.

A new 2-week laboratory course on Synthetic Biology was taught by young and dynamic faculty in the recently opened Hershey Laboratory. This emerging discipline takes inspiration from our ever-expanding ability to measure and manipulate biological systems, and the philosophical reflections of Schrodinger and Feynman that physical laws can be used to describe and rationally engineer biology to accomplish useful goals.

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor. Their excellence and dedication make the course program work exceptionally well. We would especially like to thank Drs. Richard Burgess (with more than 20 years of teaching protein chemistry at CSHL), Naomi Altman, David DiGregorio, Stacey Harmer, Sue Jaspersen, Lee Kraus, Rusty Lansford, Robert Last, Julin Maloof, Kelsey Martin, Jennifer Raymond, and Michael Zody, who all retired after many years of service.

Grants from a variety of sources support the courses. The core support provided through the recently renewed grant from the Howard Hughes Medical Institute remains critical to our course program. The courses are further supported by multiple awards from the NIH and NSF, and additional support for individual courses is provided from various foundations and other sources. The courses also depend on equipment and reagents that are loaned or donated by a large number of companies, partnerships that are invaluable in ensuring that the courses offer training in the latest technologies.

Now in its fourth year of operation, the Cold Spring Harbor Asia (CSHA) program, under the direction of Dr. Maoyen Chi, is headquartered at the Suzhou Dushu Lake Conference Center, a purpose-built academic conference center on the outskirts of old Suzhou, within a high-technology suburb (SIP). CSHA's scientific program includes large symposia and meetings, training workshops, and Banbury-style discussion meetings. It is a wholly owned subsidiary of CSHL and is not beholden to outside partners in terms of our scientific programming. In 2013, 17 scientific conferences and one summer school were held in Suzhou, predominantly attracting scientists from the Asia/Pacific region. Attendance by junior Asian scientists continues to be one of the challenges faced by the program, but the 50% growth in attendance between the first and fourth year of operations bodes well for the future. This program is described in more detail in a separate Annual Report.

Alumni Notes: The 2013 Nobel Prize in Physiology or Medicine was awarded to Randy Schekman, Jim Rothman (both alumni of the CSHL yeast course), and Tom Sudhof, a regular attendee at CSHL meetings and founding organizer of the Channels, Receptors, & Synapses meeting in 2004.

The Meetings & Courses Program staff comprises a lean team of talented professionals 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.

Terri Grodzicker
Dean of Academic Affairs

David Stewart
Executive Director,
Meetings & Courses Program/
President, Cold Spring Harbor Asia

78TH COLD SPRING HARBOR LABORATORY SYMPOSIUM ON QUANTITATIVE BIOLOGY

Immunity and Tolerance

May 29–June 3 382 Participants

ARRANGED BY **Michel Nussenzweig,** HHMI/The Rockefeller University
Anne O'Garra, MRC National Institute for Medical Research
Stephen Smale, University of California School of Medicine
David Stewart and Bruce Stillman, Cold Spring Harbor Laboratory

D. Littman, A. O'Garra

The Cold Spring Harbor Symposia on Quantitative Biology bring together scientists from all over the world to present and evaluate new data and ideas in rapidly moving areas of biological research. Each year, a topic is chosen that appears to be at a stage where general and intensive scrutiny and review are needed. There are numerous criteria for selection of topics, including the rate of progress in a given field, how recent research is highlighting connections between fundamental biological mechanisms, and the potential applications of the new discoveries to human health and disease. Cold Spring Harbor Laboratory selected the theme of Immunity and Tolerance for the historic 78th Symposium in the series.

The immune system evolved to defend individuals against invading pathogens. However, because exaggerated responses to pathogens or commensals can result in damage to the host, mechanisms of immune regulation have developed to inhibit pathology. These mechanisms also inhibit the immune response to self-antigens and so prevent autoimmune disease. Advanced molecular techniques to perturb gene function in experimental mouse models as well as the identification of genetic mutations in human diseases have provided conclusive evidence toward the role of particular molecules and pathways in vivo, in the development and function of the immune system. The genomic era has contributed to significant advances in our understanding of mechanisms underlying human disease. During the past decades, immunology research has been revolutionized by the rapid identification of molecules, pathways, and cells contributing to innate and adaptive immune responses. In addition, this has furthered our understanding of how failures in immunoregulation during immune responses to pathogens, commensals, or self can result in disease and inaugurated the era of biologics as therapeutic agents. Major advances have been made toward our understanding of the regulatory mechanisms in place to prevent inflammatory and autoimmune diseases. In turn, immunologists and clinicians are attempting to exploit this knowledge to harness these pathways in order to eradicate cancer.

The decision to focus the 2013 Symposium on immunity and tolerance reflected the enormous research progress achieved in recent years. The plan was to provide a broad synthesis of the current state of the field, setting the stage for future discoveries and application and to introduce research topics on mechanisms of human disease, extending beyond the more basic molecular research of past years. The Symposium spanned a broad range of areas of investigation including molecular mechanisms of B- and T-lymphocyte development in experimental models, at levels ranging from the single cell to the entire organism and from single genes to genomes. There was a good cover of

G. Natoli, S. Smale

J.D. Watson, M. Nussenzweig, W. Yokoyama

development and function of innate cells, including myeloid cells, NK cells, and a more recently defined innate lymphoid cell (ILC); ILC resembles T cells in producing effector molecules such as cytokines but lacks an antigen receptor and plays an early role like other innate cells in the immune response. In keeping with Cold Spring Harbor tradition, the program included talks on in-depth molecular mechanisms of transcriptional regulation of key immune pathways in innate and adaptive cells of the immune system. Emerging areas of research were also covered including how metabolic pathways affect immune responses and conversely how immune cells may affect cell and tissue physiology outside the immune response, as well as how commensal bacteria can affect the immune response contributing to protection against pathogens or disease. Diverse immune mechanisms underlying the events leading to chronic infection or immune control in HIV and tuberculosis in addition to mechanisms underlying autoimmune diseases were introduced, which have potential to lead to new clinical therapies.

In arranging the Symposium, immunologists interacted with Cold Spring Harbor organizers to introduce more on the mechanisms of the immune response underlying human disease. Opening night speakers included Anjana Rao, Jean-Laurent Casanova, Fiona Powrie, and James Allison. Of special excitement with respect to human disease was the progress that Jim Allison presented on "Immune Checkpoint Blockade in Cancer Therapy," having advanced this area by combining findings from experimental models and human disease to expedite treatments of cancer. Hidde Ploegh delivered a compelling Dorcas Cummings Lecture on "The Logic of Your Immune System" to Laboratory friends, neighbors, and Symposium participants in advance of the annual dinner parties.

This Symposium was attended by almost 385 scientists from more than 20 countries, and the program included 67 invited presentations and 200 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. In addition, interviews by Laurie Dempsey, Karin

B. Stillman, J. Allison

Dumstrel, John Inglis, Richard Sever, Liz Thompson, 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 Allergy and Infectious Diseases, a branch of the National Institutes of Health, and Merck. 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*

Development and Antigen Receptor Gene Assembly
Chairperson: S. Ghosh, *Columbia University College of Physicians & Surgeons, New York*

Gene Regulation
Chairperson: E. Rothenberg, *California Institute of Technology, Pasadena*

Microbial Immunity
Chairperson: P. Cresswell, *HHMI/Yale University School of Medicine, New Haven, Connecticut*

Anti-HIV Immunity and Vaccination
Chairperson: D. Mathis, *Harvard Medical School, Boston, Massachusetts*

NK Cells and Innate Lymphoid Cells
Chairperson: P. Marrack, *HHMI/National Jewish Center, Denver, Colorado*

Autoimmunity
Chairperson: K. Murphy, *HHMI/Washington University, St. Louis, Missouri*

Dorcas Cummings Lecture: The Logic of Your Immune System
H. Ploegh, *Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge*

Development and Function of Myeloid Subsets
Chairperson: C. Vinuesa, *Australian National University, Canberra*

Short Talks from Submitted Abstracts
Chairperson: S. Pierce, *NIAID/National Institutes of Health, Rockville, Maryland*

Tolerance, Tregs, and Inflammasome
Chairperson: R. Grosschedl, *Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany*

Tumor Immunology and Genome Stability
Chairperson: M. Davis, *HHMI/Stanford University, California*

T-Cell Differentiation and Function
Chairperson: P. Bjorkman, *HHMI/California Institute of Technology, Pasadena*

Summary
W. Paul, *NIAID/National Institutes of Health, Rockville, Maryland*

R. Medzhitov, Y. Belkaid

L. Ramakrishnan

H. von Boehmer, I. Weissman

MEETINGS

From Base Pair to Body Plan: 60th Anniversary of DNA

February 28–March 3 195 Participants

ARRANGED BY **Alex Gann,** Cold Spring Harbor Laboratory
Rob Martienssen, Cold Spring Harbor Laboratory

M. Levine, M. Ashburner

February 28, 1953, was an important day for biology. It was not the day (2 months later) that the structure of DNA was published in the journal *Nature,* nor the day (more than 10 years later) that James D. Watson, Francis Crick, and Maurice Wilkins won the Nobel Prize for perhaps the most important biological discovery of the 20th century. Rather, February 28, 1953, was the day when Watson and Crick discovered the base pair, the key chemical structure that underlies the interpretation and transmission of genetic information. On that day in 1953, the pair famously announced in the Eagle (a Cambridge pub frequented by members of the University) that they had discovered the secret of life. In celebration of the 60th anniversary of this momentous occasion, the Laboratory hosted a 4-day symposium, organized by Alex Gann and Rob Martienssen, focused on the role that the base pair has had in our understanding of developmental biology and gene regulation. Attended by six Nobel laureates and 26 speakers from around the world, the symposium included sessions on gene regulation, genome surveillance, pluripotency, stem cells, pattern formation, and evolution, chaired by such luminaries as Tom Maniatis and Brigid Hogan. The opening session (chaired by Bruce Stillman) included some fighting words from Mark Ptashne on genetic and epigenetic inheritance, as well as seminal talks on stem cells (from Nobel laureate Sir John Gurdon), and pattern formation (from Nobel laureate Christiane Nusslein Volhart). Blackford Bar was retrofitted to resemble the Eagle pub, complete with a mock ceiling from the "RAF bar," the original signed by United States and United Kingdom servicemen during WWII. The Cold Spring Harbor version is now signed by several meeting participants, including Michael Ashburner (Genetics, Cambridge). Other highlights included a session devoted to anecdotes surrounding that day in 1953, from Matthew Meselson and Nobel laureates Walter Gilbert, Sydney Brenner, and Jim Watson himself, prompted by his ironic remark in *Nature* "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material." Meeting participants were summoned to dinner by an alpine horn, courtesy of Walter Schaffner, and the symposium was topped off with a gala banquet at Oheka Castle, presided over by Robert Tjian, president of the Howard Hughes Medical Institute and Cold Spring Harbor alumnus, as well as many current and former employees and friends of the laboratory.

Funding for this meeting was provided by Life Technologies.

PROGRAM

Introduction
R. Tjian, *HHMI/University of California, Berkeley*

Gene Regulation
Chairperson: T. Maniatis, *Columbia University Medical Center, New York*

Renewal and Differentiation
Chairperson: C. Greider, *Johns Hopkins University, Baltimore, Maryland*

Special Talk
R. Tjian, *HHMI/University of California, Berkeley*

Specification and Pattern Formation
Chairperson: W. Schaffner, *University of Zurich, Switzerland*

Genome Surveillance
Chairperson: R. Martienssen, *HHMI/Cold Spring Harbor Laboratory*

Panel Discussion
S. Brenner, *Salk Institute for Biological Studies*
W. Gilbert, *Cambridge, Massachusetts*
M. Meselson, *Harvard University*
J.D. Watson, *Cold Spring Harbor Laboratory*

Evolution and Adaptation
Chairperson: B. Stillman, *Cold Spring Harbor Laboratory*

L. Caporale, J.D. Watson

S. Brenner. J.L. Sikorav, Y. Wu

T. Pederson, M. Meselson

B. Stillman, H. Ptashne

J. Gurdon, R. Tjian

Single-Cell Analyses

March 6–9 114 Participants

ARRANGED BY **Nancy Allbritton,** University of North Carolina
James Eberwine, University of Pennsylvania Medical School
Scott Fraser, California Institute of Technology

The goal of this third workshop was to bring together scientists who work with single cells using different experimental paradigms to discuss the progress that is being made. Nearly 112 scientists convened with 31 talks and 22 posters. Many cell types were discussed, including bacteria, *C. elegans,* and mammalian cells. Discussions centered around techniques used to investigate single-cell biology, including 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. Several presenters discussed particular progress in the single-cell analysis of human cells. An important component of the meeting was discussion of various imaging modalities, including light sheet and SPIM. The importance of, and necessity for, microfabrication and nanotechnology was highlighted in several talks. Finally, the multidisciplinary nature of single-cell analyses was demonstrated by the discussion of many novel single-cell-resolving biosensors that combined the expertise of molecular biologists, chemists, engineers, and biophotonics experts. The need for quantitative analysis of biological properties/processes in live cells in their natural microenvironment was a consistent theme. The 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.

This meeting was funded in part by Clontech Laboratories.

PROGRAM

Keynote Speaker
M. Chalfie, *Columbia University*

Single-Cell Transcriptomics/Genomics I
Chairperson: J. Eberwine, *University of Pennsylvania Perelman School of Medicine, Philadelphia*

Single-Cell Transcriptomics/Genomics II
Chairperson: M. McConnell, *University of Virginia School of Medicine, Charlottesville*

Single-Cell Proteomics
Chairperson: A. Vertes, *George Washington University, Washington, DC*

Microfluidics Applied to Single Cells I
Chairperson: N. Allbritton, *University of North Carolina, Chapel Hill*

Microfluidics Applied to Single Cells II
Chairperson: P. Dittrich, *ETH Zurich, Switzerland*

Imaging Advances in Analysis of Single-Cell Biology
Chairperson: S. Fraser, *University of Southern California, Los Angeles*

M. Chalfie, S. Fraser

J Eberwine, J. Morrison

A. Vertes, N. Allbritton

T. Masujima, B. Ray

Genes and Diagnostics: A Myriad of Issues in Biotech IP

March 10–13 47 Participants

ARRANGED BY **Salim Mamajiwalla,** In(sci)te IP, Markham, Canada
Rochelle K. Seide, RKS Consulting, Boca Raton, Florida

R. Seide

S. Mamajiwalla

The year 1980 was transformative for biotechnology. By a narrow decision of five to four, the United States Supreme Court, in *Diamond versus Chakrabarty*, found that Ananda Chakrabarty's patent application covering oil-digesting bacteria constituted patentable subject matter under United States patent law and paved the way for the commercialization of biotechnology to the highly dynamic industry it is today. Other countries, such as Australia, Canada, and the United Kingdom, soon followed suit and found microorganisms patentable subject matter. No one could have foreseen then the breakneck speed at which biotechnology would advance and the accompanying patents that would be granted. The Supreme Court's statement in Chakrabarty that "anything under the sun made by man" is patentable subject matter gave the USPTO license to broaden the boundaries of patentable subject matter in the life sciences and grant patents with respect to a wide variety of biotechnological innovations. Entrepreneurial scientists and patent lawyers have, in turn, tested the limits of these boundaries. Although "anything under the sun made by man" may be patentable subject matter, recent cases challenging the patentability of genes and diagnostic methods clearly suggest that the patentability of biotechnological inventions related to this subject matter is not a settled point of law, not just in the United States, but also in other jurisdictions, most notably in Europe and Australia. In the United States, the *Association for Molecular Pathology versus Myriad Genetics* and *Mayo versus Prometheus* cases rekindled the debate on the patentability of biotechnological inventions, primarily genes and diagnostic methods. Similar challenges to biotechnology patents in Europe and Australia have also reignited the debate on the patentability of such inventions in these jurisdictions.

This special Cold Spring Harbor conference was organized to explore the legal, ethical, and social issues related to the patenting of biotechnological inventions and how, if at all, these issues might influence the patentability of such inventions, and ultimately the future of the biotechnology industry as a commercial endeavor. The conference attracted prominent judges, patent lawyers, CEOs, and ethicists from all over the world. Attendees debated the impact of recent judicial decisions and future direction the judiciary in the United States, Europe, and Australia might take and how this may impact the future of the industry. Sessions addressing a variety of issues at the meeting are listed below.

The conference was funded, in part, by the generous support of Bereskin & Parr LLP, Baker Botts LLP, Jones Day LLP, Biotechnology Industry Organization (BIO), and Genentech.

PROGRAM

Pre-Conference Workshops
IP 101: Aimed at scientists, entrepreneurs, venture capitalists, and investors
BIOTECH 101: Aimed at patent practitioners, venture capitalists, and investors

Introduction and Overview
S. Mamajiwalla, *In(sci)te IP, Markham, Canada*
R.K. Seide, *RKS Consulting, Boca Raton, Florida*

Keynote Speaker
T. Caulfield, *Faculty of Law, University of Alberta, Edmonton, Canada*

Patenting Genes and Diagnostics: The Judiciary's View
Chairperson: R. Seide, *RKS Consulting, Boca Raton, Florida*

A Global Perspective on Patentable Subject Matter, Patentability, Validity, and Enforceability
Chairperson: L. Coruzzi, *Jones Day, New York*

Patenting and Biotechnology at Cold Spring Harbor Laboratory
Chairperson: S. Mamajiwalla, *In(sci)te IP, Markham, Canada*

Are Gene and Diagnostic Patents a Hindrance or Help to Industry?
Chairperson: M. Wales, *Human Genome Sciences, Rockville, Maryland*

The Impact of Prometheus on Personalized Medicine
Chairperson: W. Woessner, *Schwegman Lundberg Woessner LLP, Minneapolis, Minnesota*

Patenting Genes and Diagnostics: Policy and Ethics

Where to Next?

Meeting Review and Summary

R. Seide, M. Malathi

L. Parla, D. Kadiri

Systems Biology: Networks

March 13–16 151 Participants

ARRANGED BY **Patrick Aloy,** Institute for Research in Biomedicine, Spain
Suzanne Gaudet, Dana-Farber Cancer Institute
Trey Ideker, University of California, San Diego
Chad Myers, University of Minnesota

S. Gaudet

With a growing appreciation of the complexity of biological systems, the field of network biology has come to the forefront of life sciences in the past decade. The science of biological networks spans both descriptive work, with the systematic characterization of various interaction networks, and mechanistic investigations, probing the fundamental principles governing molecular and cellular behaviors in both health and disease. As in previous editions, this conference gathered together a growing community, with many repeat attendees and also newcomers, a group of leading minds in the world of cell networks and systems biology who presented the most promising recent advances and discussed how future developments will unfold.

This year, the program was organized into six main topics as listed below. The program opened on Tuesday with the first of two keynote addresses from Eric Schadt. His presentation beautifully highlighted the power of not just systematic, but also comprehensive characterization of a patient's biological status in the field of individualized medicine. In the following days, the program continued with 17 invited presentations and 19 short talks selected from submitted abstracts, all of them of outstanding quality. The presentations covered many aspects of network and systems biology, from methodology development, to characterization of the topology of various types of networks, to interrogation of dynamic network behaviors after genetic interventions or spontaneous mutations. Some presentations highlighted how much better we are getting at predicting the behavior of certain biological systems, and others were eye opening specifically because they emphasized how much we still have to learn.

B. Raphael, C. Myers

H. Pedersen, V. Gudmundsdottir

More than 70 abstracts were accepted for poster presentations, and the posters were divided into two groups and displayed on Wednesday and Thursday evenings, in a very relaxed atmosphere during wine and cheese mixers. These sessions were great opportunities for attendees with different skills and expertise to discuss network science and exchange ideas.

Late Saturday morning, we had the pleasure to hear the second keynote address from Arthur Lander. Dr. Lander gave what he dubbed a "Sticky Note Talk," a truly thought-provoking presentation, challenging us to remember as we move forward to think not only about the "how" of biological systems, but also about the "why." Only once we identify the objectives of biological systems—the "why"—will we be able to derive a deep mechanistic, and predictive, understanding of that system's behavior.

This meeting was funded, in part, by the National Cancer Institute and the National Human Genome Research Institute, branches of the National Institutes of Health.

PROGRAM

Keynote Speaker
E. Schadt, *Mount Sinai Medical Center, New York*

Gene Regulatory Networks
Chairperson: B. Andrews, *University of Toronto, Canada*

Chemical–Genetics Networks
Chairperson: C. Myers, *University of Minnesota, Minneapolis*

Signaling Networks
Chairperson: S. Gaudet, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

Protein–Protein Interaction Networks I
Chairperson: P. Aloy, *Institute for Research in Biomedicine, Barcelona, Spain*

Protein–Protein Interaction Networks II
Chairperson: P. Braun, *Technical University of Munich, Germany*

Metabolic and Synthetic Networks
Chairperson: M. Walhout, *University of Massachusetts Medical School, Worcester*

Networks and Disease
Chairperson: T. Ideker, *University of California, San Diego*

Keynote Speaker
A. Lander, *University of California, Irvine*

P. Braun, E. Klipp

R. Edwards, N. Sahni

Computational Cell Biology: The Interplay between Modeling and Experimentation

March 19–22 88 Participants

ARRANGED BY **Galit Lahav,** Harvard Medical School
Wallace Marshall, University of California, San Francisco
Ed Munro, University of Chicago
Vito Quaranta, Vanderbilt University

This 2013 meeting marked the exit from the organizing committee of Leslie Loew and John Tyson, the original founding members. Thank you Les and John for your foresight in creating this compelling scientific forum! To follow in their tracks, the basic format of the meeting was maintained: The opening talk of a session was given by an established leader, who also functions as chairperson, and the remaining slots are filled with speakers chosen from the submitted abstracts. To select these oral presentations, organizers and session chairs entertained lively discussion in the preceding weeks, ensuring coverage of the latest, most exciting science. As expected of a meeting tracing the interplay of experiments and modeling, all presentations intertwined computational and experimental approaches. Poster sessions were also animated by vibrant discussions around the work presented.

Two keynote addresses were delivered, respectively, by Jennifer Lippincott-Schwartz, who described advances in fluorescent reporters and super-resolution microscopy ushering a new era in structural cell biology, and by Michael Elowitz, who eloquently spoke about lessons from synthetic gene circuits. Marileen Dogterom chaired a session on Cell Mechanics, presenting work to study organization of and force generation by the cytoskeleton in cell free systems. This year, a new emphasis on disease was introduced to the meeting: A cancer systems biology theme was covered in two sessions, respectively, chaired by Vito Quaranta (substituting for a suddenly indisposed

J. Lippincott-Schwartz, G. Lahav, J. Liu

E. Munro, G. Gaglia

Michael Yaffe, MIT), who spoke about tumor cell heterogeneity captured by targeted drug-induced proliferation rates, and Sylvia Plevritis, who described computational tools to characterize differentiation-based breast cancer heterogeneity. Covered cancer topics included p53 oscillations in single cells, and control of G_1/S transition. Wendell Lim and Orion Weiner chaired the session on Synthetic Biology, with talks on maintenance of cell polarity, molecular dynamics within cells, and molecular circuitry regulating cell states. The session on Development, chaired by Angela DePace, covered fly, zebrafish, and mouse model organisms, focusing on computational models for gene regulation. The Noise and Statistical Physics session, cochaired by Guron Suel and Rob Phillips, reported on molecular and mathematical approaches to study stochastic processes in cells. Organizing Principles of Biological Systems was chaired by James Ferrell, who described feedback circuits controlling the cell cycle. Noise control in cells, as well as principles of microbial colony organization, was also discussed in this session. Imaging and Computer Vision was chaired by Richard Murphy, speaking on useful "features" for segmenting and classifying cell images in automated fashion. The session included description of computational methods to classify iPS and heterogeneity of growing cell size. A session on Signal Processing was opened by its chair, Boris Kolodenko, with a comprehensive recap of the underlying features of the EGFR network. Homeostatic controlling motifs and models for bacterial sporulation were covered as well in this session.

A steady feature of this meeting has become the improvisational theater to build a social context for conversations among scientists, cleverly led by the gifted Raquel Holmes. After some understandable hesitation, all scientists participated fervently, and a splendid time was had by all.

The meeting was supported by a grant from the National Science Foundation as well as additional contributions from various companies. The meeting will observe its biennial schedule (March/April 2015). NSF representative Gregory W. Warr enthusiastically participated at the meeting and confirmed that it is among the NSF's priorities for continued support.

PROGRAM

Keynote Speaker
J. Lippincott-Schwartz, *National Institutes of Health*

Cell Mechanics: Shape, Motility, and Cytoskeleton
Chairperson: M. Dogterom, *FOM Institute AMOLF, Amsterdam, The Netherlands*

Cancer Systems Biology I
Chairperson: M. Yaffe, *MIT, Broad Institute, Cambridge, Massachusetts*

Synthetic Biology
Chairpersons: W. Lim and O. Weiner, *University of California, San Francisco*

ImprovScience Session
Chairperson: R. Holmes, *University of Connecticut Health Center, Farmington*

Keynote Speaker
M. Elowitz, *California Institute of Technology, Pasadena*

Development
Chairperson: A. DePace, *Harvard Medical School, Boston, Massachusetts*

Noise and Statistical Physics in Cells
Chairpersons: G. Suel, *University of California,* San Diego; R. Phillips, *California Institute of Technology, Pasadena*

Organizing Principles of Biological Systems
Chairperson: J. Ferrell, *Stanford University, California*

Imaging and Computer Vision of Cells
Chairperson: R. Murphy, *Carnegie Mellon University, Pittsburgh, Pennsylvania*

Signal Processing, Circuit Design, and Decision Making
Chairperson: B. Kholodenko, *Systems Biology Institute, Dublin, Ireland*

Cancer Systems Biology II
Chairperson: S. Plevritis, *Stanford University School of Medicine, California*

C. Carmona-Fontaine, O. Marchenko, W. Marshall

E. Stukalin, C. Lopez

RNA and Oligonucleotide Therapeutics

April 10–13 120 Participants

ARRANGED BY **Ryszard Kole,** Sarepta Therapeutics
Adrian Krainer, Cold Spring Harbor Laboratory
Arthur Krieg, RaNA Therapeutics
Bruce Sullenger, Duke University

R. Kole

This inaugural Cold Spring Harbor conference 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 and microRNAs 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 from 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.

The meeting opened with a keynote address by Nobel laureate Dr. Sydney Altman. The sessions addressed modulation of RNA splicing, antisense oligonucleotides, posttranscriptional control of gene expression, siRNA, miRNA, and aptamers as therapeutics, delivery of RNA therapeutics, and immunomodulating oligonucleotides.

The topic of the meeting attracted funding from several companies; the main sponsor: AVI Biopharma. This meeting was funded, in part, by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health; Isis Pharmaceuticals; and Sarepta Therapeutics.

A. Krainer, S. Dowdy

T. Haraguchi, A. Krieg

PROGRAM

Keynote Speakers
J. Lee, *Massachusetts General Hospital, Boston;*
V.J. Dzau, *Duke University, Durham, North Carolina*

Aptamers
Chairperson: L. Gold, *SomaLogic, Inc., Boulder, Colorado*

Clinical Development of ASOs and SSOs
Chairperson: G. Campion, *Prosensa, Leiden, The Netherlands*

Therapeutic Modulation of RNA Processing
Chairperson: R. Kole, *Sarepta Therapeutics, Bothell, Washington*

Immunomodulatory Oligos/RNAs
Chairperson: E. Gilboa, *University of Miami, Florida*

Noncoding RNAs
Chairperson: A. Krieg, *RaNA Therapeutics, Cambridge, Massachusetts*

Delivery
Chairperson: L. Sepp-Lorenzino, *Merck, West Point, Pennsylvania*

A. Aartsma-Rus

M. Huang, A. Fiorillo

E. Phimister

Synapses: From Molecules to Circuits and Behavior

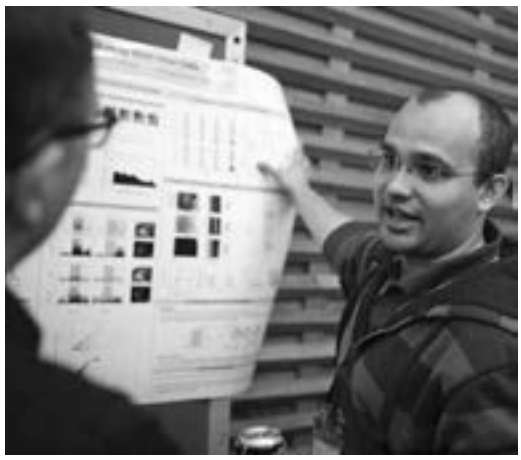
April 17–20 159 Participants

ARRANGED BY **Hollis Cline,** The Scripps Research Institute
Michale Fee, Massachusetts Institute of Technology

The brain consists of a vast network of specialized cells, neurons, and glia, which communicate with each other through specialized contacts, called synapses. The nervous system processes and stores information encoded as patterns of electrical activity. As master regulators of neuronal excitability and synaptic communication, ion channels and receptors lie at the heart of neurobiology. All aspects of nervous system function, including perception, cognition, and action, depend on accurate information processing by synapses, neurons, and networks. 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 the study of neuronal and circuit plasticity one of the most rapidly growing in neuroscience. In contrast to previous years, scientists seeking understanding of mechanisms of circuit and behavioral plasticity in healthy or neuropathophysiological conditions are expanding their range of vision past the previous focus of synaptic pathologies to examine network modifications arising from epigenetic and synergistic effects on multiple nodal points within brain machinery. This year's meeting saw a more diverse representation of experimental animals in use to investigate brain plasticity, which represents increased appreciation of the lessons that evolution can contribute to neuroscience.

This fifth very successful meeting brought together nearly 200 participants from the United States, Europe, and Asia in an atmosphere of social and scientific exchange. A wide range of topics (listed below) were discussed, with an emphasis on the biophysics and cell biology of synapses and neurons, the integration of 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. The meeting was opened by an outstanding plenary lecture given by Dr. Richard Tsien.

K. Haas, E. Ruthazer, H. Cline



A. Banerjee

R. Tsien, G. Popescu

T. Venkatesh, P. Garrity

The overall response to the meeting was extremely positive, continuing to cement the reputation of this Cold Spring Harbor conference in the field. Attendees enjoyed the breadth of subjects covered and the chance to interact with investigators in related but distinct fields. The unusual opportunity for junior investigators (including postdocs and students) to present their own work was welcomed.

The meeting was supported with funds from the National Institute on Drug Abuse and the National Institute of Mental Health, branches of the National Institutes of Health.

PROGRAM

Keynote Speaker
R. Tsien, *Stanford University, California*

Ion Channels, Receptors, and Transporters
Chairpersons: P. Garrity, *Brandeis University, Waltham, Massachusetts;*
L. Looger, *Howard Hughes Medical Institute, Ashburn, Virginia*

Synapse Formation
Chairpersons: S.-H. Shi, *Memorial Sloan-Kettering Cancer Center, New York;*
Y. Dan, *University of California, Berkeley*

Synaptic Plasticity
Chairpersons: R. Yasuda, *Max-Planck Florida Institute for Neuroscience, Jupiter;*
A. Barth, *Carnegie Mellon University, Pittsburgh, Pennsylvania*

F. Albeanu, K. Podgorski

D. Beacham, S. Herlitze

Circuit Development
Chairpersons: C. Aizenman, *Brown University, Providence, Rhode Island;*
T. Mrsic-Flogel, *University College London, United Kingdom*

Neuronal and Circuit Plasticity
Chairpersons: J. Wang, *University of California, San Diego;*
S. Herlitze, *University of Bochum, Germany*

Circuit and Behavior Plasticity
Chairpersons: F. Engert, *Harvard University, Cambridge, Massachusetts;*
L. Stowers, *Scripps Research Institute, La Jolla, California*

Behavioral Plasticity
Chairpersons: G. Feng, *Massachusetts Institute of Technology, Cambridge;*
C. Murphy, *Princeton University, New Jersey*

Diseases of Synapses and Circuits
Chairpersons: A. Muotri, *University of California, San Diego;*
H. Song, *Johns Hopkins University, Baltimore, Maryland*

I. Miracourt, E. Pastrana

T. Kennedy, C. Cho

Cancer Biology and Therapeutics

April 23–27 244 Participants

ARRANGED BY **Senthil Muthuswamy,** Ontario Cancer Institute
Kornelia Polyak, Dana-Farber Cancer Institute
David Tuveson, Cambridge Research Institute

J. Allison, S. Muthuswamy

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 each other, developing ways to image the changes in a tumor, and exploring therapeutic opportunities that take into account the noncancer cells. Thus, there is an emergence of a need for an integrative approach for controlling cancer. This meeting brought together molecular and computational biologists who are decoding the cancer genome and epigenome; biochemists studying cell metabolism; physicists developing ways to image cells and tumors; immunologists developing ways to harness the immune system; cell biologists identifying new ways to stop the growth and spread of tumor cells; mouse modelers developing models to study human cancers; and medical oncologists using all of this information to best treat patients. Including the keynote speaker Bill Kaelin, several prominent cancer researchers who are pioneers and thought leaders in their fields presented and participated in the meeting. In addition, we held a lively panel discussion titled "Will the ineffectiveness of oncology trials be rescued by personalized medicine?" In this provocative session chaired by clinicians and scientists, we attempted to identify the real barriers to implementing superior cancer care, including scientific, fiscal, and logistical limitations. The meeting, as a whole, was received very well, and the participants enjoyed both the diversity and the depth of topics covered.

R. Jain, S. Mohla, D. Tuveson

A. Giannakou, N. Rosen

This meeting was funded, in part, by the National Cancer Institute, a branch of the National Institutes of Health; and Astellas Pharma.

PROGRAM

Keynote Speaker
W.G. Kaelin, *HHMI/Dana-Farber Cancer Institute, Boston, Massachusetts; Brigham & Women's Hospital, Boston, Massachusetts.*

Tumor Heterogeneity I: Genetic
Chairpersons: E. Mardis, *Washington University School of Medicine, St. Louis;*
M. Wigler, *Cold Spring Harbor Laboratory*

Diagnostic Imaging and Drug Delivery
Chairpersons: R. Jain, *Massachusetts General Hospital, Boston;*
K. Brindle, *University of Cambridge, United Kingdom*

Immunotherapy
Chairpersons: J. Allison, *University of Texas MD Anderson Cancer Center, Houston;*
B. Vonderheide, *Abramson Family Cancer Research Institute, Philadelphia, Pennsylvania*

Translational Trial/Personalized Cancer Therapy
Chairpersons: L. Siu, *Princess Margaret Hospital, Toronto, Canada;*
L. Norton, *Memorial Sloan-Kettering Cancer Center New York*

Panel Discussion
Will the Ineffectiveness of Oncology Trials Be Rescued by "Personalized Medicine"? Does This Mean Better Integration with Preclinical Studies, Better Trial Designs, Better Training for Caregivers, and Safer Drugs?

Tumor Heterogeneity II: Epigenetic
Chairperson: M. Brown, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Tumor Cell Plasticity and Microenvironment
Chairpersons: B. Stanger, *University of Pennsylvania, Philadelphia;*
V. Weaver, *University of California, San Francisco*

Metabolism
Chairperson: J. Brugge, *Harvard Medical School, Boston, Massachusetts*

Signaling Networks and Therapeutics
Chairpersons: N. Rosen, *Memorial Sloan-Kettering Cancer Center, New York;*
L. Cantley, *Weill Cornell Medical College/New York Presbyterian Hospital, New York*

S.-C. Bae, Y. Ito

R. Parmigiani, C. Attwooll

Telomeres and Telomerase

April 30–May 4 331 Participants

ARRANGED BY **Titia de Lange,** The Rockefeller University
Roger Reddel, Children's Medical Research Institute
Virginia Zakian, Princeton University

The conference consisted of eight talk sessions 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. Session chairs were given the choice between giving a 12-min presentation themselves or having a member of their lab give a talk. The rest of the presentations (also 12 min) were chosen from submitted abstracts, allowing as many presentations as possible. These presentations were primarily by graduate students and postdoctoral fellows. Attendance exceeded 300 participants, a high fraction of whom presented the 161 posters and 82 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. Most of the presented data were unpublished 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 2015.

This meeting was funded, in part, by the National Institute on Aging, a branch of the National Institutes of Health.

D. Frescas, A Sfeir, N. Bosco, I. Schmutz, S. Kabir, Y. Doksani

PROGRAM

Yeast Telomere Function/CST Structure and Function
Chairpersons: J. Cooper, *Cancer Research, London, United Kingdom;*
T. Nakamura, *University of Chicago, Illinois*

ALT and Telomerase Structure/Biochemistry
Chairpersons: R. Wellinger, *Université de Sherbrooke, Canada;*
T. Cech, *HHMI/University of Colorado, Boulder*

Telomerase Structure and Biochemistry
Chairpersons: K. Collins, *University of California, Berkeley;*
M. Blasco, *CNIO, Madrid, Spain*

Telomerase Recruitment and Regulation
Chairpersons: C. Price, *University of Cincinnati, Ohio*;
C. Greider, *Johns Hopkins University, Baltimore, Maryland*

Consequences of Telomere Dysfunction
Chairpersons: L. Harrington, *Institut de Recherche en Immunologie et Cancérologie, Montréal, Canada;* M. Godinho Ferreira, *Instituto Gulbenkian de Ciência, Oeiras, Portugal*

Terra and Mammalian Telomere Function I
Chairpersons: W. Wright, *University of Texas Southwestern Medical Center, Dallas;*
C. Azzalin, *ETH, Zurich, Switzerland*

Mammalian Telomere Function II
Chairpersons: D. Shippen, *Texas A&M University, College Station;*
J. Karlseder, *The Salk Institute, La Jolla, California*

C. Nelson, S. Bailey

Telomeres and Disease
Chairpersons: S. Savage, *National Cancer Institute, Rockville, Maryland;*
E. Gilson, *University of Nice, France*

C. Wagner, S. Stanley

D. Shore, B. Luke

J. Alder, A. Garbuzo

The Biology of Genomes

May 7–11 **558** Participants

ARRANGED BY **Ewan Birney**, EBI/EMBL
Carlos Bustamante, Stanford University
Joel Hirschhorn, The Broad Institute of Harvard/MIT
Elaine Mardis, Washington University School of Medicine

This meeting marked the 26th annual gathering of genome scientists at Cold Spring Harbor Laboratory. A total of 558 investigators from around the world attended the meeting, with more than 385 abstracts presented describing a broad array of topics relating to the production, analysis, and interpretation of genomes from diverse organisms. The meeting built upon the remarkable progress in the sequencing, functional annotation, and analysis of genomes from many human individuals, "model organisms," other animals including new genomic studies of deer mice, *Drosophila*, and the very challenging large conifer genomes. There were talks from the 1000 Genomes Project and other genome projects. 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, as well as other species. Many groups are generating transcriptome, transcription-factor-binding site and epigenomic maps to understand transcriptional complexity and regulation. There were interesting talks on the history of humans, both more recent history (e.g., in Africa) and early human history (e.g., population inference of early hominid populations).

All sessions (listed below) were well attended, stretching the capacity of the CSHL facilities. Andrew Fire and Eric Lander gave the keynote presentations.

The ELSI (Ethical, Legal, and Social Implications) panel was moderated by Hank Greely; the area of discussion was "Ethical Issues in Noninvasive Prenatal Testing (NIPT) and Other Advances in Prenatal Testing Technology."

This meeting was funded, in part, by the National Human Genome Research Institute, a branch of the National Institutes of Health; Complete Genomics, Fluidigm, Illumina, Ingenuity Systems, and Sunrise Scientific.

M. Kellis, E. Lander

F. Yu, K. Vaydylevich

M. Cullen, D. Kural

PROGRAM

High-Throughput Genomics and Genetics
Chairpersons: M. Blaxter, *University of Edinburgh, United Kingdom;*
M. Snyder, *Stanford University, California*

Genetics of Complex Traits
Chairpersons: M. Daly, *Massachusetts General Hospital/Broad Institute, Boston;*
C. Willer, *University of Michigan, Ann Arbor*

ENCODE Tutorial
Chairpersons: M. Snyder, *Stanford University, California;*
M. Pazin, *NHGRI;* J. Stamatoyannopoulos, *University of Washington*

Functional and Cancer Genomics
Chairpersons: L. Pennaccio, *Lawrence Berkeley National Laboratory, California;* B. Wold, *California Institute of Technology, Pasadena*

Computational Genomics
Chairpersons: J. Korbel, *European Molecular Biology Laboratory, Heidelberg, Germany;*
Y. Song, *University of California, Berkeley*

ELSI Panel and Discussion: Ethical Issues in Noninvasive Prenatal Testing (NIPT) and Other Advances in Prenatal Testing Technology
Moderator: H. Greely, *Stanford University Panelists:*
R. Wapner, *Columbia University Medical Center;*
B. Berkman, *National Human Genome Research Institute;*
M. Cho, *Stanford University;*
S. Chandrasekharan, *Duke University*

Evolutionary Genomics
Chairpersons: D. Kingsley, *Stanford University, California;*
H. Hoekstra, *Harvard University, Cambridge, Massachusetts*

Genetics and Genomics of Nonhuman Species
Chairpersons: M. Przeworski, *University of Chicago, Illinois;*
M. Nordborg, *Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria*

Keynote Speakers
Chairpersons: A. Fire, *Stanford University School of Medicine;*
E. Lander, *The Broad Institute of MIT and Harvard*

Population Genomic Variation
Chairpersons: A. Siepel, *Cornell University, Ithaca, New York;*
J. Kidd, *University of Michigan Medical School, Ann Arbor*

M. Blaxter, K. Worley

N. Yutin, T. Hubbard

M. Nordborg, D. Schwartz

The Ubiquitin Family

May 14–18 247 Participants

ARRANGED BY **Ronald Hay,** University of Dundee
Ron Kopito, Stanford University
Brenda Schulman, St. Jude Children's Research Hospital

R. Ray, B. Shulman, R. Kopito

This fifth meeting following the successful inauguration of this series in 2003 focused on ubiquitin and a group of structurally related "ubiquitin-like" proteins and their roles in regulation of various cellular processes. Major questions in the field include how specificity in ubiquitin and ubiquitin-like protein conjugation reactions is maintained, the molecular mechanisms by which specific ubiquitin chain linkages are recognized and regulate distinctive pathways, roles of the ubiquitin system in physiology and disease, and opportunities and mechanisms for therapeutically targeting the ubiquitin system. These questions are being elegantly addressed using structural biology, sophisticated kinetic studies, systematic library screening, and quantitative proteomics technologies by an increasing number of investigators in the field. In addition, important advances continue to be made in understanding how ubiquitin and its family members contribute to the operation of diverse cellular pathways, and how these functions are perturbed in diseases ranging from viral infections to cancers. Exciting progress is also being made in the development of small-molecule inhibitors of a range of enzymes involved in ubiquitin and ubiquitin-like protein pathways.

The meeting this year attracted 247 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, highlighted by recent cryo-EM structures of the 26S proteasome in action. There have been tremendous advances in understanding the involvement of ubiquitin quality control mechanisms used by cells to eliminate otherwise toxic misfolded proteins, in terms of characterizing disease-related ubiquitination machineries such as the Parkin E3 ligase, and a wide range of distinctive quality control pathways converging on ubiquitin. 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 of ubiquitin and ubiquitin-like proteins in diverse physiological processes ranging from control of stem cell division to DNA repair and pathways associated with diseases such as neurological disorders and cancers. Other highlights of the meeting this year concerned progress on use of mass spectrometry both to define the ubiquitin and SUMO-modified proteomes and to 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. 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; this includes the first presentation of structures of RBR E3 ligases as well as autoinhibited conformations of Parkin and mechanisms underlying ubiquitin ligation by HECT E3s. From a technology standpoint, a new "ubiquitin chain sequencing" methodology, through use of DUBs in the OTU pathway with distinctive specificities, will greatly enable the field. There was

also a great increase in understanding assembly and functions of linear ubiquitin chains and other forms of protein amino-terminal ubiquitination. 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 understand the regulatory mechanisms by which ubiquitin and ubiquitin-like proteins function, their ever-growing roles in cellular physiology and disease, and development of therapeutics targeting the ubiquitin family.

R. Tomko, Jr.

This meeting was funded, in part, by the National Cancer Institute, the National Institute of Child Health and Human Development, and the National Institute of General Medical Sciences, branches of the National Institutes of Health; Boston Biochem; and Ubiquigent.

PROGRAM

Conjugation, Removal, and Recognition of Ubiquitin and Ubiquitin-Like Proteins I
Chairpersons: D. Komander, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom;* R. Klevit, *University of Washington, Seattle*

Keynote Speaker
M. Hochstrasser, *Yale University, New Haven, Connecticut*

Conjugation, Removal, and Recognition of Ubiquitin and Ubiquitin-Like Proteins II
Chairpersons: C. Lima, *Memorial Sloan-Kettering Cancer Institute, New York;*
M. Rape, *University of California, Berkeley*

Substrate Targeting and Degradation
Chairpersons: D. Finley, *Harvard Medical School, Boston, Massachusetts;*
A. Martin, *University of California, Berkeley*

Quality Control
Chairpersons: C. Joazeiro, *The Scripps Research Institute, La Jolla, California;*
R. Hampton, *University of California, San Diego*

UBLs in Signaling I: Receptors, Plasma Membrane/Endosomal Trafficking
Chairpersons: S. Polo, *European Institute of Oncology, Milan, Italy;*

J. Kowalski, C. Dahlberg

A. Fox

F. Melchior, C. Lima

I. Dikic, *Goethe University Medical School, Frankfurt, Germany*

Nuclear Functions of Ubiquitin-like Proteins
Chairpersons: C. Wolberger, *HHMI/Johns Hopkins University School of Medicine, Baltimore, Maryland;* F. Melchior, *University of Heidelberg, Germany*

Regulation of Intracellular Pathways
Chairpersons: J. Huibregtse, *University of Texas, Austin;*
H. Ulrich, *Cancer Research UK, London Research Institute*

Keynote Speaker
J.W. Harper, *Harvard Medical School, Boston, Massachusetts*

UBLs in Signaling II: Intracellular Communication and Regulation of Organismal Biology
Chairpersons: R. Deshaies, *HHMI/California Institute of Technology, Pasadena;*
T. Sixma, *Netherlands Cancer Institute, Amsterdam*

J. Corn, L. Frappier

Retroviruses

May 20–25 396 Participants

ARRANGED BY **Frank Kirchhoff,** Ulm University Medical Center, Germany
Reuben Harris, University of Minnesota

A total of 396 scientists from 23 countries attended this 5-day 2013 Cold Spring Harbor meeting, which featured 120 talks and 179 poster presentations covering a broad range of topics. More than 38% of the participants were female scientists, and 229 (57.8%) were junior trainees (graduate or postdoctoral level). Like many retrovirologists, the organizers have contributed to this meeting for many years. This meeting is highly interactive and forms the basis for many long-term friendships and collaborations.

The first keynote lecture was given by Dr. Warner Greene, who has made key contributions to our understanding of the transmission and pathogenesis of HIV/AIDS. Dr. Greene's talk addressed the long-standing question of how HIV-1 induces the depletion of $CD4^+$ T cells that represents a key feature of AIDS. He reported the surprising finding that most T cells in ex vivo HIV-infected human lymphoid tissues are abortively infected and that cell death is induced by caspase 1 and due to pyroptosis, a process associated with high levels of inflammation. Dr. Greene also suggested that available caspase-1 inhibitors may help to reduce the immune damage caused by HIV-1, which could be an adjuvant line of treatment in addition to combinatorial antiretroviral therapy (ART). His presentation resulted in lively discussions and numerous questions that kept him on stage long after the allotted hour.

The second keynote speaker was Dr. Ben Berkhout, who has long-standing expertise in retroviral transcription, RNA structure, and antiretroviral agents. His presentation touched upon many issues, among others on the striking finding that HIV-1 can actually become dependent on a drug, that is, the entry inhibitor T20. Furthermore, Dr. Berkhout reminded us that the HIV-1 genome has a very unusual composition characterized by a high percentage of "A" nucleotide. He also presented results suggesting that inducible live-attenuated SIV constructs exert protective effects in the rhesus macaque model and may offer a novel avenue for the development of an AIDS vaccine.

A. Hulme, J. Sastr, Z. Lukic

The meeting was organized into 10 oral and three poster sessions covering the various steps of the viral life cycle, mechanisms of virus adaptation to different hosts, as well as viral immune evasion and persistence. Most presentations dealt with HIV with a particular focus on host restriction factors and their viral antagonists. As usual for this meeting, mainly novel unpublished data were presented, and sufficient time was allowed for discussion between sessions. Three prizes were awarded at this meeting: Sharon Schlesinger received the Andy Kaplan young faculty prize for her studies on proviral silencing; Judd F. Hultquist was decorated with the Ute von Schwendler prize for the best Ph.D. research in retrovirology and reported his results on the role of CBFβ in counteraction of APOBEC3G by the viral Vif protein. Finally, Siddarth Venkatesh received the best poster award for his studies on the mechanisms of HIV-1 entrapment by tetherin.

J. Lingappa, E. Arts

Several presentations described how HIV and SIV adapt to their respective hosts. One report described strong selective sweeps in HIV-1-infected individuals that are most likely driven by the immune response. Others indicated that both TRIM-5α and APOBEC3 proteins drive species-specific adaptations that increase viral fitness in primate lentiviruses. Furthermore, it was reported that the creation of SIVcpz resulted in the loss of the *vpx* gene and that vpu-containing SIVs may cause disease in their natural simian hosts. Several talks focused on the regulation of viral latency, and one highlight was the finding that Yin Yang 1 may induce proviral silencing in embryonic cells.

Restriction factors were once again a major topic in several sessions. Emerging evidence suggests that some of these antiviral factors, such as tetherin and TRIM5, also have important roles in viral immune sensing, and the underlying mechanisms were discussed in several talks. Furthermore, some novel potential restriction factors, for example, MxB, IFITMs, and Schlafen 1, were presented. Three oral presentations reported that the antiretroviral activity of SAMHD1 is regulated by phosphorylation. Two studies in mouse models support that SAMHD1 exerts antiviral and anti-inflammatory activity in vivo. Another report showed that Vpr and Vpx have evolved multiple means to target SAMHD1, once again highlighting the complexity and plasticity of virus–host interactions. Notably, several studies suggested that inhibition of reverse transcription activity by reduction of intracellular dNTP levels may not be the only mode of SAMHD1-dependent retroviral restriction.

L. Mansky, S. Goff

S. Hughes, V. Kewal Ramani

Z. Williams

B. Liu, A. Cheatham, T. Turner, H. Taylor

The APOBEC/Vif axis figured prominently at the meeting with the largest number of abstracts of any category (reflected as cover of the symposium abstract book). Highlights on this topic included in vivo data from humanized and transgenic mice, cats, and monkeys, as well as cell-based studies on mechanisms of RNA binding, virion encapsidation, and deamination target site selection. A high-resolution structure of APOBEC3F catalytic domain was presented, as was a low-resolution structure of the Vif ubiquitin ligase complex, with optimism for higher-resolution larger complexes in the near future. APOBEC3F was also shown to have the surprising capacity to ride with the viral core from budding, through entry and reverse transcription, and even into the nuclear compartment. More work will be necessary to tie this unexpected property to mechanism, but APOBEC3F may still be a powerful tool for cell biology of the early steps of virus replication.

The last session of the meeting focused on nuclear entry and integration. Highlights included the unexpected observation that several integration-blocking compounds also cause a late-stage maturation defect. Several talks also focused on the integrity of the viral capsid during reverse transcription up to the stage of nuclear import. Other presentations described the pathway(s) of nuclear import, with several studies highlighting CPSF6, TNPO3, and nuclear pore components. A TNPO3 structure was reported with RAN-GDP and GTP. Participants were very optimistic that the stages of virus replication related to integration are good targets for additional antiretroviral drug development.

This meeting was funded, in part, by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health; and Gilead Sciences.

PROGRAM

Latency and Evolution
Chairpersons: S. Goff, *Columbia University, New York*;
S. Sawyer, *University of Texas, Austin*

Seventh Annual Andy Kaplan Prize
Awarded by: A. Telesnitsky, *University of Michigan, Ann Arbor*
To: S. Schlesinger, *Columbia University, New York*

Virus and Host Gene Expression
Chairpersons: E. Browne, *Massachusetts Institute of Technology, Cambridge*;
J.-C. Paillart, *CNRS-Université de Strasbourg, France*

Assembly and RNA Packaging
Chairpersons: M. Johnson, *University of Missouri, Columbia;*
R. Craven, *Pennsylvania State University College of Medicine, Hershey*

Keynote Speaker
W. Greene, *Gladstone Institute, University of California, San Francisco*

Budding, Maturation, and BST2/Tetherin
Chairpersons: T. Hatziioannou, *Aaron Diamond AIDS Research Center, New York;*

J. Stoye, J. Luban

D. Evans, J. Guatelli

D. Evans, *Harvard Medical School, Southborough, Massachusetts*

Transmission and Entry
Chairpersons: W. Mothes, *Yale University, New Haven, Connecticut;*
A. Finzi, *Centre de Recherche du CHUM, Université de Montréal, Canada*

RT, Mutagenesis, and APOBECs
Chairpersons: M.-A. Langlois, *University of Ottawa, Canada;*
V. Simon, *Icahn School of Medicine at Mount Sinai, New York*

Second Annual Ute Von Schwedler Prize for Retrovirology
Awarded by: V. Vogt, *Cornell University*
To: J.F. Hultquist, *University of Minnesota, Twin Cities*

RT, dNTPs, and SAMHD1
Chairpersons: N. Laguette, *CNRS UPR1142, Montpellier, France;*
F. Diaz-Griffero, *Albert Einstein College of Medicine, Bronx, New York*

Keynote Speaker
B. Berkhout, *University of Amsterdam*, *The Netherlands*

RT, Uncoating, Trim5, and Novel Host Factors
Chairpersons: K. Bishop, *MRC National Institute for Medical Research, London, United Kingdom;* E. Campbell, *Loyola University, Chicago, Illinois*

PICs, Nuclear Entry, and More Restriction Factors
Chairpersons: V. Kewal Ramani, *National Cancer Institute, Frederick, Maryland;*
W. Johnson, *Boston College, Chestnut Hill, Massachusetts*

Third Annual Daniel Wolf Prize
Awarded by: Stephen Goff, *Columbia University, New York*
To: Jury-selected poster presenter at the 2013 Retroviruses meeting

Integration
Chairpersons: P. Cherepanov, *Cancer Research UK/Imperial College London, United Kingdom;*
J. Luban, *University of Massachusetts Medical School, Worcester*

Wiring the Brain

July 18–22 195 Participants

ARRANGED BY **Catalina Betancur,** INSERM, Paris
Ed Bullmore, Cambridge University
Josh Huang, Cold Spring Harbor Laboratory
Helen Mayberg, Emory University
Kevin Mitchell, Trinity College Dublin, Ireland

This was the first Wiring the Brain conference hosted at Cold Spring Harbor, following two previous meetings in this series held in Ireland in 2009 and 2011. The main goal of this meeting is to bring together researchers from diverse fields to explore how brain connectivity is established, how genetic variation can affect these processes, how circuit and network function are affected by defects in neural development, and how this can lead to psychiatric and neurological disease. The program was designed with a particular focus on neurodevelopmental disorders and covered a wide range of areas relevant to this theme.

Session topics are listed below. There were 28 invited talks from world leaders in diverse fields and an additional 20 talks selected from submitted abstracts. These talks highlighted cutting-edge research in specific fields, and it was also notable how many of them were integrating across levels of analysis, using new technologies to transition smoothly from cells to circuits to systems, from development to function or dysfunction, and between animals and humans.

Two stimulating keynote lectures set an excellent tone for the meeting. The first, by Huda Zoghbi, described her long-running work on Rett syndrome, a single-gene disorder manifesting as autism. This was an inspirational talk, going from careful clinical observation and definition of the syndrome to gene discovery, to elucidation of molecular and neurobiological pathogenic mechanisms, and ultimately to the design and development of therapies now in clinical trials. The second talk was by Edmund Rolls, who presented a computational perspective on brain wiring, asking what neural circuits are designed to do and how their anatomy and connectivity is constrained by these functional imperatives.

There were also 87 posters, which were similarly outstanding in the quality of research presented, again across very diverse fields. Poster sessions were very well attended, lively, and stimulating.

G. Marcus, K. Mitchell, E. Viding, S. Fisher

A. Zuko, M. Kas, K. Kleijer

The meeting was characterized by a very open attitude and wide-ranging discussions centered on big questions, rather than focused on technical details. As such, it provided a unique forum for the integration of research from developmental neurobiology, psychiatric genetics, molecular, cellular, and systems neuroscience, cognitive science, and psychology. These kinds of interactions will help build an integrative explanatory framework in neuroscience, especially in our understanding of the nature and causes of brain disorders.

This meeting was funded, in part, by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health; and Forest Laboratories.

PROGRAM

Genetically Programmed Development
Chairpersons: K. Mitchell, *Trinity College Dublin, Ireland;*
Z.J. Huang, *Cold Spring Harbor Laboratory*

Activity-Dependent Development
Chairpersons: Z.J. Huang, *Cold Spring Harbor Laboratory;*
H. Mayberg, *Emory University, Atlanta, Georgia*

Genetics of Neurodevelopmental Disorders
Chairpersons: C. Betancur, *INSERM, Paris, France;*
K. Mitchell, *Trinity College Dublin, Ireland*

Keynote Speakers
H.Y. Zoghbi, *HHMI/Baylor College of Medicine;*
E. Rolls, Jan and Duncan Neurological Research Institute, Texas Children's Hospital

O. Bibollet-Bahena, D. Shanley

D. Geschwind, J. Korenberg

Modeling Neurodevelopmental Disorders
Chairpersons: Z.J. Huang, *Cold Spring Harbor Laboratory;*
C. Betancur, *INSERM, Paris, France*

Connectivity: From Synapses to Systems
Chairpersons: E. Bullmore, *University of Cambridge, United Kingdom;*
H. Mayberg, *Emory University, Atlanta, Georgia*

Circuits: Function and Dysfunction
Chairpersons: Z.J. Huang, *Cold Spring Harbor Laboratory;*
H. Mayberg, *Emory University, Atlanta, Georgia*

Wiring the Human Brain
Chairpersons: C. Betancur, *INSERM, Paris, France;*
E. Bullmore, *University of Cambridge, United Kingdom*

Keynote Speaker
E. Rolls, *Oxford Centre for Computational Neuroscience; University of Warwick, Coventry*

Cognitive Development: The Emergence of the Mind
Chairpersons: H. Mayberg, *Emory University, Atlanta, Georgia;*
K. Mitchell, *Trinity College Dublin, Ireland*

C. Ross, D. Lewis

Metabolic Signaling and Disease: From Cell to Organism

August 13–17 209 Participants

ARRANGED BY **Daniel Kelly,** Sanford-Burnham Medical Research Institute, Lake Nona
Mitchell Lazar, University of Pennsylvania
Susanne Mandrup, University of Southern Denmark

M. Lazar, S. Mandrup, D. Kelly

This meeting was conceived following a highly successful Symposium on Quantitative Biology 2 years earlier. The main goal of the meeting was to bring together researchers from diverse fields to explore how principles of cellular metabolism manifest in different cell types, including cancer; how differences in metabolic regulation underlie the functions of differentiated tissues; and how these differences impact on organismal physiology and disease. The meeting was subtitled "From Cell to Organism" to highlight these objectives.

The 26 invited speakers were leaders in the various aspects of metabolic research from all over the world. A keynote address by Nobel laureate Michael Brown set a superb tone on the opening evening. A total of 10 sessions followed, all highlighting unpublished research. Eight sessions featured oral presentations. Of these, two focused on metabolic cells and their role in storing, converting, and sharing metabolites; three sessions emphasized signaling mechanisms, metabolic networks, and organ–organ cross-talk. An additional session concentrated on the physiology and pathophysiology of energy metabolism, whereas the final two sessions focused on altered metabolism in aging and disease states including cancer, obesity and diabetes. Short talks were chosen from abstracts to increase the exposure of younger investigators and to highlight hot topics that complemented and extended the exciting program.

The total number of abstracts was 153, including 127 posters that were presented in two sessions. All 10 sessions were characterized by open and wide-ranging discussions, and the meeting provided a unique forum for the exploration of the commonalities and differences in metabolic principles and details across different laboratories, systems, and diseases. Indeed, a great success

of the meeting was that attendees gained in-depth exposure to the amazing cell, organ, and disease specificity of metabolic flux and its regulation.

This meeting was funded, in part, by the National Institute of Diabetes and Digestive and Kidney Diseases, a branch of the National Institutes of Health.

PROGRAM

Keynote Speaker
M. Brown, *University of Texas Southwestern Medical School, Dallas*

Professional Metabolic Cells I: Storing Metabolites
Chairperson: T. Osborne, *Sanford-Burnham Medical Research Institute at Lake Nona, Orlando, Florida*

Professional Metabolic Cells II: Converting and Sharing Metabolites
Chairperson: B. Kahn, *Beth-Israel Deaconess Medical Center, Boston, Massachusetts*

Signaling Metabolism I: Cellular Signaling Networks
Chairperson: D. Muoio, *Duke University, Durham, North Carolina*

Signaling Metabolism II: Metabolite Signals
Chairperson: M. Birnbaum, *University of Pennsylvania, Philadelphia*

Signaling Metabolism III: Organ-Organ Cross-Talk
Chairperson: R. Evans, *Salk Institute for Biological Studies, La Jolla, California*

G. Hardie, J.D. Watson

Burning Metabolites: Energy and Heat
Chairperson: K. Schoonjans, *Ecole Polytechnique Federale de Lausanne, Switzerland*

Cellular Proliferation and Metabolic Adaptation: Cancer
Chairperson: D. Moore, *Baylor College of Medicine, Houston, Texas*

Metabolic Failure: Aging and Metabolic Disease
Chairperson: C. Thompson, *Memorial Sloan-Kettering Cancer Center, New York*

R. Evans, N. Hah

N. Prior, S. Borrego

Eukaryotic mRNA Processing

August 20–24 336 Participants

ARRANGED BY **Tom Blumenthal,** University of Colorado
Kristen Lynch, University of Pennsylvania
Karla Neugebauer, Yale University

K. Neugebauer, T. Blumenthal, K. Lynch

This meeting was held in order to present and discuss 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. Structural, live-cell imaging, and genome-wide approaches were interwoven into the sessions, reflecting state-of-the-art techniques currently applied to understanding how mRNA is processed in vivo and in vitro. Following on our aim to include all aspects of RNA biology, reports on miRNA, RNAi, and noncoding RNA were encouraged and well represented.

In addition to our traditional splicing mechanisms session, this topic was further visited in our kick-off session on RNA–protein interactions as well as a session on RNP assembly and functions. One highlight among splicing mechanisms was the presentation of a high-resolution structure of the yeast PRP24 protein bound to the U6 snRNA, revealing extensive interfaces of the protein chaperone with the RNA that promote RNA rearrangements en route to mature U6 snRNP formation. These phenomena contrasted with energy-dependent rearrangements carried out by, for example, Prp5 and Prp16 in the spliceosome, as well as a study on the role of U6 snRNA at the catalytic center.

This year, an overwhelming number of abstracts on alternative splicing regulation were received, reflecting the development of approaches to studying transcript isoforms in model organ-

Crossing Airslie lawn

isms and tissues. This also reflects the momentum the field has gained in identifying RNA–protein interactions, thanks largely to in vivo cross-linking approaches such as CLIP. Therefore, many abstracts on alternative splicing also appeared in the sessions on RNA–protein interactions and RNP assembly and functions, in addition to the session on RNA processing and disease and global analyses. These included the development of splicing targeted therapeutics.

L. Guilgur, M. Gonzalez-Porta

Several presentations on alternative 3′-end formation established that poly(A) site choice is highly regulated and a major source of transcript variability, linking polyadenylation, transcription, and quality control. A major advance in the field, namely, the discovery of triple-helix formation at 3′ ends, providing resistance to mRNA decay mechanisms, was described in the last session. In the RNA decay session, the nonsense-mediated decay (NMD) pathway was a particular focus, including work showing that NMD sculpts the cellular transcriptome, disconnecting transcript abundance from transcriptional output, by altering the stability of specific transcripts. Several studies homed in on the activity of Staufen, a double-stranded RNA-binding protein implicated in Staufen-mediated decay (SMCD), in order to understand how target specificity is achieved.

The RNA Cell Biology and Cotranscriptional RNA-processing sessions focused on genome-wide approaches to RNA–protein interactions that influence RNA localization, live-cell imaging, NGS approaches to measuring the kinetics of transcription and splicing in living cells, and changes in processing due to cell signaling and progression through the cell cycle.

This meeting was funded, in part, by the National Cancer Institute, a branch of the National Institutes of Health; and the National Science Foundation.

PROGRAM

RNA–Protein Interactions

Chairpersons: J. Doudna, *HHMI/University of California, Berkeley;* A. Zahler, *University of California, Santa Cruz*

RNP Assembly and Functions

Chairpersons: R. Lührmann, *Max-Planck Institute of Biophysical Chemistry, Göttingen, Germany;* M. Jurica, *University of California, Santa Cruz*

B. Graveley, B. Blencowe

J. Witkowski, J. Wiluz

H. Lou, E. Lasda

S. Sharma, M. Peterson

Cotranscriptional RNA Processing
Chairpersons: N. Proudfoot, *University of Oxford, United Kingdom;* T. Johnson, *University of California, San Diego*

Mechanisms of RNA Splicing/Global Analyses and Evolution
Chairpersons: M. Sattler, *Helmholtz Zentrum München, Neugerberg, Germany;* C. Guthrie, *University of California, San Francisco*

RNA Cell Biology
Chairpersons: A.G. Matera, *University of North Carolina, Chapel Hill;* M. Carmo-Fonseca, *University of Lisbon, Portugal*

Translational Control/Decay Mechanisms
Chairpersons: R. Parker, *HHMI/University of Colorado, Boulder;* N. Conrad, *University of Texas Southwestern Medical Center, Dallas*

RNA Processing in Development and Disease
Chairpersons: M. Ares, *University of California, Santa Cruz;* A. Krainer, *Cold Spring Harbor Laboratory*

miRNA Biogenesis and Function/ncRNA Processing
Chairpersons: F. Gebauer, *Center for Genomic Regulation, Barcelona, Spain*; E. Lund, *University of Wisconsin, Madison*

Mechanisms of Eukaryotic Transmission

August 27–31 367 Participants

ARRANGED BY **Stephen Buratowski,** Harvard Medical School
Katherine A. Jones, The Salk Institute for Biological Studies
John Lis, Cornell University

J. Lis, K. Jones, S. Buratowski

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 and genomics. This 13th Cold Spring Harbor meeting appropriately covered many aspects of the field and brought together a diverse group of scientists. It consisted of eight plenary sessions, two poster sessions, and one breakout session.

The meeting began with a session that included exciting new insights derived from structure and imaging studies of mammalian, yeast, and archaeal RNA polymerase preinitiation complexes. The second session covered new mechanisms that control factor targeting and activation of transcription initiation. The next plenary session described mechanisms that activate chromatin remodeling and actin-containing complexes during transcription, and new mechanisms that regulate transcription in response to phosphorylation, signaling or differentiation. The fourth session introduced several new factors and complexes that regulate RNAPII elongation and coupled events, such as cotranscriptional splicing, nucleosome modification, termination, and mRNA export. The next plenary session continued with the elongation theme but was primarily focused on enzymatic complexes that modify the RNAPII carboxy-terminal domain (CTD). Additional topics covered in this session include transcriptional control by mRNA capping enzymes, gene looping, and a role for alternative termination

Y. Ghavi-Helm, M. Werner

C. Engel, F. Werner

complexes in premature termination at specific genes. The sixth and seventh sessions described the role of histone chaperones, variant histones, noncoding RNAs and novel complexes that read histone marks in regulating transcription and establishing euchromatin or heterochromatin domains in development and disease. The final session included genome-wide approaches for understanding how gene networks are organized and regulated through superenhancers, promoter structure, and long-range chromatin interactions and for understanding how chromatin and dynamics are interrelated.

Interspersed with these oral presentations were the two poster sessions where a wide variety of exciting unpublished transcriptional research was presented. The meeting also including a breakout session, initiated at the previous meeting and focused on complexes that recognize distinct phosphorylation patterns of the RNAPII CTD. Breakout sessions provide a unique forum to focus on rapidly evolving topics, and this one was well attended. The organizers plan to continue this session organization in future meetings on topics yet to be determined.

This meeting was funded, in part, by the National Science Foundation.

PROGRAM

Initiation Complexes
Chairperson: P. Cramer, *Gene Center LMU Munich, Germany*

Activators and Co-Activators
Chairperson: J. Conaway, *Stowers Institute for Medical Research, Kansas City, Missouri*

Signaling and Regulatory Complexes
Chairperson: K. Arndt, *University of Pittsburgh, Pennsylvania*

Elongation, Pausing, and Termination
Chairperson: W. Bickmore, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

The CTD and Postinitiation Events
Chairperson: R. Young, *Whitehead Institute, MIT, Cambridge, Massachusetts*

Transcriptionally Active Chromatin
Chairperson: A. Shilatifard, *Stowers Institute for Medical Research, Kansas City, Missouri*

Repressive Chromatin
Chairperson: K. Luger, *HHMI/Colorado State University, Fort Collins*

Genomics and Systems Biological Approaches
Chairperson: B. Cairns, *Huntsman Cancer Institute, University of Utah, Salt Lake City*

N. Fuda, R. Young

A. Ansari, D. Reinberg

Behavior and Neurogenetics of Nonhuman Primates

September 6–9 54 Participants

ARRANGED BY **Nelson Freimer,** University of California, Los Angeles
Jeffrey Rogers, Baylor College of Medicine

N. Freimer, J. Rogers

This inaugural meeting provided an opportunity to bring together investigators from different fields marked by rapid recent progress, including genetics, genomics, and neuroimaging. The meeting sessions all related clearly to the overall topic, but emphasized distinct aspects of this broad field.

The first session introduced topics related to the genomics of brain function and behavior. The second session focused on primate brain evolution, incorporating perspectives ranging from neuroanatomy to comparative gene expression. The keynote address, by Svante Pääbo, was a tour de force presentation. He used a comprehensive genomic comparison between extinct human species and modern humans, including exciting unpublished data, as a framework for understanding the evolution of the primate brain and behavior. The keynote provided an excellent bridge to the third session, which focused on the genetics of behavior in free-ranging or wild primates. As in other areas of biology, technology developments promise to transform investigations of primate brain and behavior. Integrating new technologies into various aspects of primate research, including studies of wild or free-ranging animals, was a recurrent theme of the conference. The presentations in session four showed the value of novel methods in primate research clearly. They described a series of approaches that are not feasible in humans or where non-human primates provide better models for human disease than do rodents. These talks included the use of transgenic technologies to model human diseases in monkeys, the introduction of optogenetics in non-human primate species, and the application of functional and structural brain imaging in a primate model of anxiety. Session five focused on brain

C. Dehay, K. Kosik, M. Arcila

development, continued the theme of non-human primate models, focusing on regional differences in gene function at critical developmental time points, the role of microRNAs, and interspecies differences as assessed through transcriptional network analyses. The final session focused on non-human primate models of disease-related processes including stress response, temperament, and activity patterns. The poster session covered a diverse set of topics and stimulated considerable discussion. A principal goal of the meeting was to provide a unique venue for investigators who do not overlap at other meetings to make new acquaintances and initiate communications. In an open-discussion session that ended the meeting, attendees enthusiastically reported that this goal had been achieved, and they endorsed the view that the CSHL conference format had played an important part in this achievement. In this discussion, the attendees voiced strong support for future CSHL meetings on the topics covered in this meeting.

This meeting was funded, in part, by the National Institutes of Health.

U. Borello, T. Nowakowski

PROGRAM

Introductory Session
Chairpersons: J. Rogers, *Baylor College of Medicine;* N. Friemer, *University of California, Los Angeles*

Genome Structure and Brain Function
Chairpersons: E. Eichler, *University of Washington, Seattle;* L. Stubbs, *University of Illinois, Urbana*

Comparative Evolution of the Brain
Chairpersons: P. Khaitovich, *CAS-MPG Partner Institute for Computational Biology, Shanghai, China;* E. Lein, *Allen Institute for Brain Science, Seattle, Washington*

Keynote Speaker
S. Pääbo, *Max-Planck Institute for Evolutionary Anthropology and Biology, Leipzig, Germany*

Evolution and Behavior
Chairpersons: S. Alberts, *Duke University, Durham, North Carolina;* A. Varki, *University of California, San Diego*

New Technologies
Chairpersons: K. Deisseroth, *Stanford University, California;* E. Sasaki, *Keio University, Tokyo, Japan*

Neurodevelopment and Stem Cell Biology
Chairpersons: C. Dehay, *INSERM, Bron, France;* D. Geschwind, *University of California, Los Angeles*

Genetics of Primate Behavior
Chairpersons: J. Cameron, *University of Pittsburgh, Pennsylvania*; J. Rogers, *Baylor College of Medicine, Houston, Texas*

Concluding Remarks and Discussion
J. Rogers, *Baylor College of Medicine, Houston;* N. Freimer, *University of California, Los Angeles*

Eukaryotic DNA Replication and Genome Maintenance

September 9–13 297 Participants

ARRANGED BY **Anindya Dutta,** University of Virginia
Joachim Li, University of California, San Francisco
Johannes Walter, Harvard Medical School

A. Dutta, J. Walker, J. Li

This fourth biannual meeting reflected the remarkable progress that has been made in the past 2 years and serves an important role in bringing together researchers studying eukaryotic DNA replication, repair, and their interactions to maintain genome stability. As in the past, this 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 80 investigators participated in the 10 scientific sessions, with a total of 249 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 findings presented.

Sessions and their chairs at the meeting are listed below. The presentations confirmed the power of biochemical reconstitution and structural studies in dissecting the mechanisms of cellular DNA replication initiation and repair. Genome-wide and replication-fork-specific assays are beginning to reveal that origin selection in metazoan cells is probably not sequence specific, whereas replication timing is determined significantly by the status of the chromatin. Novel activities involved in termination of DNA replication were described. The abundance of specific replication proteins was shown to be important for mid-blastula transition during development. The connection between loss of replication control and gene amplification was highlighted. 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 travel support for two European investigators was provided by EMBO.

PROGRAM

New Approaches and Perspectives
Chairpersons: A. van Oijen, *University of Groningen, The Netherlands;* K. Labib, *Cancer Research UK, University of Manchester, United Kingdom*

Origin Selection and Licensing
Chairpersons: M. Aladjem, *NCI, National Institutes of Health, Bethesda, Maryland;* S. Bell, *HHMI/Massachusetts Institute of Technology, Cambridge*

Chromatin and Replication
Chairpersons: A. Groth, BRIC, *University of Copenhagen, Denmark;* Z. Zhang, *Mayo Clinic, Rochester, Minnesota*

Origin Activation and Timing
Chairpersons: H. Araki, *National Institute of Genetics, Mishima, Japan;* H. Masukata, *Osaka University, Japan*

Replisome Activities and Functions
Chairpersons: L. Bloom, *University of Florida, Gainesville;* A. Donaldson, *University of Aberdeen, United Kingdom*

Dealing with Blocks and Lesions
Chairpersons: W. Yang, *NIDDK, National Institutes of Health, Bethesda, Maryland;* P. Burgers, *Washington University School of Medicine, St. Louis, Missouri*

G. Alvino, C. Muller

B. Stillman, C. Speck

J. Sequeira-Mendes, J.-C. Cadoret

Development, Cell Cycle, and Cancer
Chairpersons: B. Calvi, *Indiana University, Bloomington;* V. Gorgoulis, *University of Athens Medical School, Greece*

Replication Stress and Damage Signaling
Chairpersons: D. Cortez, *Vanderbilt University School of Medicine, Nashville, Tennessee;* J. Lukas, *University of Copenhagen, Denmark*

Microbial Pathogenesis and Host Response

September 17–21 264 Participants

ARRANGED BY **Andrew Camilli,** Tufts University School of Medicine
Lalita Ramakrishnan, University of Washington
Malcolm Whiteway, Concordia University

M. Whiteway, L. Ramakrishnan, A. Camilli

Despite advances in modern health care, infectious diseases continue to be major causes of human morbidity and mortality. The evolution of microbial pathogens with humans has resulted in complex interactions that impact the struggle between the infectious invader and the susceptible host. Increased understanding of these interactions with the goal of developing new therapeutics and preventive strategies requires collaborative and interdisciplinary scientific approaches. This meeting brought together a diverse group of international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives. Investigators from the disciplines of molecular microbiology, eukaryotic cell biology, immunology, and genomics, and representing academia, industry, and the public health sector, shared recent findings concerning microbial and host aspects of infectious diseases.

The meeting focused on bacterial and fungal pathogens and the host response and defense to these invading microbes. Oral sessions were topic-based and included studies of multiple and diverse organisms. Areas covered included the microbial–host interactions; genetic approaches to studying microbial pathogenesis; regulation of commensalism and pathogenesis; the host inflammatory response; microbial communication and biofilms in infection; immune killing; chemical genetic approaches to studying pathogenesis; development of new antibiotics; and role of the gut microbiota in human health and disease. Speakers for each session were a mixture of established

leaders in the field and young investigators. Half of the speakers, including postdoctoral fellows and graduate students, were chosen from submitted abstracts. Dr. Jeffrey I. Gordon, the Dr. Robert J. Glaser Distinguished University Professor and Director of the Center for Genome Sciences & Systems Biology at Washington University, presented the keynote address. Dr. Gordon is an internationally recognized leader in the field of biomedical research. His presentation, "The Human Microbiome in Health and Disease," addressed how the gut microbiota is established, the factors that affect the species composition of the microbiota, the diversity between individuals but stability within individuals, and the causal role of the gut microbiota in human health.

The informal atmosphere, combined with the broad perspectives of the meeting participants, resulted in a free flow of novel and refreshing ideas on pathogenesis and clinical treatment, with the atmosphere of a small meeting. Active questioning and discussion followed all oral presentations, was evident throughout the posters sessions, and continued during a wine and cheese reception and other social gatherings. We strongly encouraged submission of abstracts by junior researchers in the field, and many young investigators were in attendance. Some of these interactions have already produced fruitful scientific collaborations.

This meeting was partly supported by funds from the National Institute of Dental and Craniofacial Research and the National Institute of Allergy and Infectious Diseases.

PROGRAM

Host Cell/Pathogen Interactions
Chairperson: C. Roy, *Yale University, New Haven, Connecticut*

Antibiotics, Chemical Genetics, and Immune Killing
Chairperson: G. Wright, *McMaster University, Hamilton, Canada*

Regulation of Commensalism/Pathogenesis
Chairperson: T. Koehler, *University of Texas Health Science Center, Houston*

Genetic Approaches to Pathogenesis
Chairperson: R. Valdivia, *Duke University Medical Center, Durham, North Carolina*

Biofilms, Quorum Sensing, and Disease
Chairperson: P. Singh, *University of Washington, Seattle*

Keynote Speaker
J. Gordon, *Washington University, St. Louis*

L. Burrows, M. Machner

A. Arbues, C. Astarie-Dequeker

A. Guzman, J. Barroso-Batista

Stem Cell Biology

September 24–28 203 Participants

ARRANGED BY **Konrad Hochedlinger,** Harvard Medical School
Fiona Watt, King's College London
Ting Xie, Stowers Institute for Medical Research

F. Watt, K. Hochedlinger

This third biannual CSHL conference was organized in thematic topics, including somatic stem cells and regeneration, stem cell niches and asymmetric cell division, pluripotency and transdifferentiation, lineage differentiation, stem cells and therapy, cancer and stem cells, and stem cells in model organisms. There were 203 participants, which is comparable to previous years (190 in 2011 and 240 in 2009). About 20% of participants were Ph.D. students, and 30% were postdocs from 23 different countries (64% from the United States), documenting the influence of the meeting on trainees from around the world. Moreover, representatives from major publishing houses such as *Cell* and *Nature* were present. Speakers comprised a mix of internationally known leaders in the individual disciplines as well as emerging junior researchers, who presented exciting unpublished work or work that had recently appeared in print. In addition, each section had one short talk that was picked from abstracts by the organizers. It was decided to increase the short talks for the next meeting (2015) to give more trainees the opportunity to present their work. Main talks were 20–25 min plus 5–10 min of discussion (28 speakers), and the short talks were 15 min plus 5 min of discussion (seven speakers). The keynote lecture was given by Rudolf Jaenisch, who presented recent work by his lab on CRISPR/Cas genome engineering in stem cells and studies on MECP2 and Rett syndrome. Discussions were very stimulating and long and often continued at poster sessions and during lunch, dinner, or at the bar. Preliminary (informal) feedback by students, postdocs, speakers, and journal editors was uniformly positive. Attendees liked the mixed topics of sessions and the inclusion of presentations on the therapeutic

I. Jordens, M. Khurgel

C. Lillyhook, E. Ezhkova

potential of stem cells (e.g., a talk by a Pfizer researcher) and model organisms in some sessions (e.g., *C. elegans, Drosophila*), which distinguish this meeting from other, more focused stem cell conferences.

This meeting was funded, in part, by Fluidigm.

PROGRAM

Somatic Stem Cells and Tissue Regeneration
Chairperson: F. Watt, *King's College London, United Kingdom*

Stem Cell Niches/Asymmetric Stem Cell Division
Chairperson: C. Blanpain, *Université Libre de Bruxelles, Brussels, Belgium*

Stem Cells in Model Organisms
Chairperson: T. Xie, *Stowers Institute for Medical Research, Kansas City, Missouri*

Pluripotency and Cellular Plasticity
Chairperson: K. Hochedlinger, *Harvard Medical School, Boston, Massachusetts*

Stem Cells in Disease Modeling and Therapy
Chairperson: R. Jaenisch, *Whitehead Institute for Biomedical Research, Cambridge, Massachusetts*

Lineage Determination
Chairperson: J. Rajagopal, *Harvard Stem Cell Institute, Massachusetts General Hospital, Boston*

Keynote Speaker
R. Jaenisch, *Whitehead Institute for Biomedical Research, Cambridge, Massachusetts*

Cancer and Stem Cells
Chairperson: J. Wysocka, *Stanford University, California*

J. Kimble, E. Matunis, B. Ohlstein

S. Wood, J. Knoblich

Neurobiology of *Drosophila*

October 1–5 427 Participants

ARRANGED BY **Tom Clandinin,** Stanford University
Linda Restifo, University of Arizona

T. Clandinin, L. Restifo

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: neural development, neural circuits and function, simple and complex behaviors, disease models, synaptic structure and function, and emerging technologies. A small fraction of abstracts submitted in each of these areas was 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 address basic questions of nervous system development and function. Among the highlights of the meeting were the creative ways in which researchers are using *Drosophila* to understand the molecular and cellular underpinnings of many different physiological and pathological processes. A focal point in the meeting was the keynote address, the Benzer Lecture, by S. Lawrence (Larry) Zipursky. Dr. Zipursky, a prominent leader in the field of *Drosophila* neurobiology and genetics, presented an historical perspective on *Drosophila* visual system development, describing 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, by the fly, and the design of new technology. A significant shift was clear

Banquet toast

in that many of the talks concerned circuits and behavior, rather than development and studies of sensory systems. The Elkins Award plenary lecture was presented by Dr. Jan Melom (now at Stanford University) based on her extraordinary doctoral dissertation research at MIT in the lab of Troy Littleton. The environment of the meeting allowed many opportunities for informal discussions among all participants. The high quality of the presentations, the ongoing development of novel techniques, and the exciting new directions of *Drosophila* research toward circuits and behavior, as well as disease models, demonstrate the vitality of this interdisciplinary research area. Discussions at the meeting led to cross-fostering of ideas that was valuable to everyone in the field.

This meeting was funded, in part, by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health.

PROGRAM

Brain, Behavior, and Evolution
Chairperson: K. Siwicki, *Swarthmore College, Pennsylvania*

Neural Circuits
Chairperson: G. Turner, *Cold Spring Harbor Laboratory*

Synapses
Chairperson: N. Reist, *Colorado State University, Fort Collins*

Elkins Memorial Lecture: Visualizing Calcium: Insights into Quantal Synaptic Transmission and Cortex Glial Function
J. Melom, *Massachusetts Institute of Technology, Cambridge*

Neural Development
Chairperson: W. Grueber, *Columbia University, New York*

Neuroscience Medley
Chairperson: L. Restifo, *University of Arizona, Tucson*

Seymour Benzer Lecture
L. Zipursky, *HHMI/University of California, Los Angeles*

Higher-Order Brain Function
Chairperson: U. Heberlein, *HHMI/Janelia Farm Research Campus, Ashburn, Virginia*

L. Zipursky, J. Simpson

Disease Models
Chairperson: A. Schenk, *Radboud University Nijmegen, The Netherlands*

Technology
Chairperson: M. Nitabach, *Yale University School of Medicine, New Haven, Connecticut*

E. Storkebaum, H. Scholz, A. Schenck

Cell Death

October 8–12 246 Participants

ARRANGED BY **Douglas Green,** St. Jude Children's Research Hospital
Sally Kornbluth, Duke University
Scott Lowe, Memorial Sloan-Kettering Cancer Center

This 10th meeting was regarded by many present as perhaps the best cell death meeting in years. More than 50 outstanding presentations and lively discussions centered on topics in the field of cell death (see list below).

D. Green, S. Kornbluth

The keynote lectures were given by Karen Vousden, who spoke on p53 and the control of metabolic stress in cell death and survival, and David Andrews, who presented cutting-edge research on the function of Bcl-2 proteins in controlling the mitochondrial pathway of apoptosis. Other highlights: Shige Nagata presented on the mechanisms of phosphatidylserine externalization in response to calcium and during apoptosis; Vishva Dixit spoke on the unexpected lethal effects of a kinase-dead RIPK3 mutation that promotes apoptosis via caspase-8; Guy Salvesen presented on the protease clan that includes the caspases, and how related biochemistry interfaces with cell death pathways; Loren Walensky spoke on the design of therapeutic peptides controlling Bcl-2 interactions not only in cell death, but also glucokinase control in diabetes; Andreas Strasser presented stunning developmental analysis of defects caused by the elimination of the three pro-apoptotic effectors, Bax, Bak, and Bok, and how these contribute, or not, to different tissues; Tony Letai talked about the application of detection of mitochondrial "priming" for apoptosis as a biomarker for cancer therapy; Junying Yuan presented new data on inhibitors of necroptosis via blockade of RIPK1; and Peter Sorger presented systems analysis of single-cell studies on caspase activation leading, or not, to cell death. Many other talks explored extensive progress in interrogating the functions of the Bcl-2 proteins, the interface of metabolism and cell death, and the functions of cell death in development, cancer, and immunity, among other topics. By limiting the total number of talks, discussion remained lively and did not have to be curtailed, and sessions were completed more or less on time. Many commented that this made the meeting less stressful than previous meetings, and more productive in terms of exploration of new ideas.

L. Bouchier-Hayes, B. Murphy

A total of 140 posters were also presented in two sessions, one held traditionally in the afternoon prior to the wine and cheese reception, and another held during the morning. The morning session attracted very active discussion and essentially complete attendance, suggesting that this approach might be continued in future meetings.

In retrospect, it should not be surprising that the field of cell death remains enormously exciting and vibrant more than 20 years after its "rebirth" in the early 1990s. Cell death is a fundamental biological process, and as we gain insights into its mechanisms,

V. Dixit, T. Mak

we unveil new processes and phenomena with deep implications. The therapeutic control of cell death, as presented at the meeting, has entered the clinic with striking effects, and we suspect this is just the beginning. The next meeting will be even better, we are sure.

This meeting was funded, in part, by the National Cancer Institute and the National Institute on Aging, branches of the National Institutes of Health.

PROGRAM

Keynote Speakers
D. Green, *St. Jude Children's Research Hospital, Memphis, Tennessee;* K. Vousden, *Beatson Institute for Cancer Research, Bearsden, United Kingdom;* D. Andrews, *Sunnybrook Research Institute, University of Toronto, Canada*

Inflammation/Immunity
Chairpersons: V. Dixit, *Genentech Inc., South San Francisco, California;* S. Nagata, *Kyoto University, Japan*

Molecular Mechanisms I
Chairperson: H. Wu, *Boston Children's Hospital, Harvard Medical School, Massachusetts*

Metabolism
Chairpersons: T. Mak, *Ontario Cancer Institute, Toronto, Canada;* J. Opferman, *St. Jude Children's Research Hospital, Memphis, Tennessee*

K. Lindblom, K. Cocce

S. Joshi, G. Liccardi

BCL-2 Family Get-Together I
Chairperson: L. Walensky, *Dana-Farber Cancer Institute, Boston, Massachusetts*

BCL-2 Family Get-Together II
Chairperson: A. Strasser, *Walter & Eliza Hall Institute of Medical Research, Parkville, Australia*

Model Organisms
Chairpersons: J.M. Hardwick, *Johns Hopkins University, Baltimore, Maryland;* K. White, *Massachusetts General Hospital, Harvard Medical School, Boston*

Cancer
Chairpersons: J. Abrams, *University of Texas Southwestern Medical School, Dallas;* S. Kornbluth, *Duke University School of Medicine, Durham, North Carolina*

Targets and Therapies
Chairpersons: S. Lowe, *Memorial Sloan-Kettering Cancer Center, New York*; E. White, *Rutgers University/The Cancer Institute of New Jersey, New Brunswick*

Nonapoptotic Cell Death
Chairpersons: D. Green, *St. Jude Children's Research Hospital, Memphis, Tennessee;* J. Yuan, *Harvard Medical School, Boston, Massachusetts*

Molecular Mechanisms II
Chairperson: G. Salvesen, *Sanford-Burnham Medical Research Institute, La Jolla, California*

Survival Strategies
Chairpersons: S. Martin, *Trinity College Dublin, Ireland;* R. Youle, *NINDS, National Institutes of Health, Bethesda, Maryland*

A. Dbrot, M. Miura

History of Restriction Enzymes

October 19–21 99 Participants

ARRANGED BY **Herbert Boyer,** University of California, San Francisco
Stuart Linn, University of California, Berkeley
Mila Pollock, Cold Spring Harbor Laboratory
Richard Roberts, New England BioLabs

This lively and productive meeting was organized by Herb Boyer, Stu Linn, Rich Roberts, and Mila Pollock. Herb Boyer is a restriction enzyme and recombinant DNA pioneer and cofounder of Genentech; Stu Linn is a restriction enzyme pioneer and professor at the University of California at Berkeley; Rich Roberts is a restriction enzyme and gene splicing pioneer, Nobel laureate, and chief scientific officer of New England BioLabs; and Mila Pollock is the executive director of the CSHL Library & Archives. This was the fifth international meeting on the history of science co-organized by the CSHL Library & Archives. It was the first to bring together the scientists who were involved with the discoveries and research on restriction enzymes dating back to the 1950s and covering developments to the present time.

The meeting was attended by more than 150 scientists, history of science scholars/authors, educators, students, and members of the CSHL community. It included sessions on the beginnings of the field, the growth of the restriction enzyme field, the development of restriction enzymes into commercial reagents, cloning and sequencing of restriction modification (RM) systems, the biochemistry of restriction enzymes, and structural studies of restriction enzymes.

A highlight of the meeting was a special toast to meeting co-organizer Rich Roberts, a presentation titled, Restriction Enzymes: Between Nature, Culture, and Politics, by historian of science and medicine and author Bruno Strasser. Other highlights included a poster session with poster presentations by nine restriction enzyme research groups and one historian of science/author; short contributions, including reminiscences of life in the restriction enzymes research community, by Robert Yuan, Lise Raleigh, David Dryden, Ichizo Kobayashi, and others; and a summarization of the meeting by Stu Linn, which included commentary on the importance of meetings,

B. Stillman, H. Smith

S. Linn, M. Meselson

B. Strasser, J.D. Watson

collaborations, and short visits to other labs; the importance of "small science;" and "some hot and future items" for restriction and modification studies.

What are restriction enzymes? Discovered in 1970, restriction enzymes cleave DNA at specific recognition sites and have many uses in molecular biology, genetics, and biotechnology. More than 4000 restriction enzymes are known today, of which more than 620 are commercially available. The 1978 Nobel Prize in Physiology or Medicine was awarded jointly to Werner Arber, Dan Nathans, and Hamilton Smith for the discovery of "restriction enzymes and their application to problems of molecular genetics." Werner Arber and Hamilton Smith both participated in the CSHL meeting. (Dan Nathans passed away in 1999.)

Major support for this meeting was provided by Life Technologies, New England BioLabs, and ThermoFisher. Significant support was provided by Genentech, Nippon, Promega, and TAKARA/Clontech. Additional support was provided by Molecular Biology Resources.

PROGRAM

The Beginnings of the Field
Chairperson: S. Linn, *University of California, Berkeley*

The Restriction Enzyme Field Begins to Grow
Chairperson: D. Dryden, *University of Edinburgh, United Kingdom*

Restriction Enzymes Become Commercial Reagents
Chairperson: S. Chandrasegaran, *Johns Hopkins University, Baltimore*

A. Creager, B. Ames

J. Josephsen, D. Dryden

Conversations: Commercialization

Cloning and Sequencing of RM Systems
Chairperson: H. Smith, *J. Craig Venter Institute, La Jolla, California*

The Biochemistry of Restriction Enzymes
Chairperson: V. Nagaraja, *Indian Institute of Science, Bangalore, India*

Structural Studies
Chairperson: Herb Boyer, *University of California, San Francisco*

Short Contributions and Summary
Chairperson: R. Roberts, *New England BioLabs, Ipswich*

M. Pollock, J. Rosenberg, H. Boyer

R. Roberts, T. Kelly

Genome Informatics

October 30–November 2 305 Participants

ARRANGED BY **Jennifer Harrow,** Wellcome Trust Sanger Institute
Michael Schatz, Cold Spring Harbor Laboratory
James Taylor, Emory University

J. Harrow, M. Schatz, J. Taylor

This 13th Cold Spring Harbor Laboratory/ Wellcome Trust conference was held at Cold Spring Harbor, New York. The conference series continues to demonstrate a robust attendance, both from the United States and from abroad. It attracted more than 300 registrants presenting 208 abstracts and offered a snapshot of the latest developments in the field. There were 12 invited talks and two keynote addresses. The remaining 36 talks were all selected for presentation by session chairs from openly submitted abstracts.

This year, abstracts covered a wide variety of genomic analyses, with a special emphasis on population-wide analysis across multiple cell types, individuals, and species driven by the massively increased availability of sequence data. There was also a focus on emerging biotechnologies for single-molecule analysis and other biochemical assays. The sessions in the conference are listed below.

The first keynote address was delivered by Dr. Michael Snyder, who focused on the possibilities enabled by detailed longitudinal multiomics assays of individuals, exemplified by his own medical records that he calls the "Synderome." He described how he has regularly had his transcriptional and metabolic profiles recorded for several years, leading him to adjust his diet and lifestyle to


B. Rao, K. Rao (father and son)


S. Pai, E. Mukamel, L. Carmel

combat the prediabetic state that he was developing. The second keynote was delivered by Dr. Lior Pachter, whose keynote address, titled "Stories from the Supplement," emphasized the need for rigorous computational and analytic methods for analyzing genomic data, with an emphasis on the challenges in transcriptome profiling. Both talks were extremely well regarded, with much follow-up discussion.

This meeting was also one of the first CSHL meetings to be completely open on Twitter (using hashtag #gi2013). More than 2000 messages were broadcasted by users around the world to discuss and debate the ideas presented.

This meeting was funded, in part, by the National Human Genome Research Institute, a branch of the National Institutes of Health.

PROGRAM

Databases, Data Mining, Visualization, and Curation
Chairpersons: C. Bult, *The Jackson Laboratory, Bar Harbor, Maine;* M. Fiume, *University of Toronto, Canada*

Keynote Speaker I
M. Snyder, *Stanford University School of Medicine, California*

Transcriptomics, Alternative Splicing, and Gene Predictions
Chairpersons: C. Burge, *Massachusetts Institute of Technology, Cambridge;* M. Stanke, *University of Greifswald, Germany*

Sequencing Pipelines and Assembly
Chairpersons: I. Korf, *University of California, Davis;* A. Quinlan, *University of Virginia, Charlottesville*

Comparative and Evolutionary Genomics
Chairpersons: J. Ma, *University of Illinois, Urbana-Champaign;* H. Roest Crollius, *Ecole Normale Supérieure, Paris, France*

Epigenomics and Noncoding Genome
Chairpersons: R. Lister, *University of Western Australia, Perth;* A. Marques, *University of Oxford, United Kingdom*

Keynote Speaker II
L. Pachter, *University of California, Berkeley*

Population and Personal Genomics
Chairpersons: Y. Erlich, *Whitehead Institute, Cambridge, Massachusetts;* D. MacArthur, *Massachusetts General Hospital, Boston*

J. Trinh, N. Pearson

J. Davila, H. Fang

Cell Biology of Yeasts

November 5–9 178 Participants

ARRANGED BY **Martha Cyert,** Stanford University
Daniel Lew, Duke University
Kenneth Sawin, University of Edinburgh

K. Sawin, M. Cyert, D. Lew

This inaugural conference is a direct descendant of the Yeast Cell Biology meeting, and it can be considered the 15th biannual international meeting devoted to diverse aspects of cell biology in yeasts. However, the slight name change reflects a new emphasis on expanding beyond the traditional focus on baker's yeast to include other ascomycetes, particularly fission yeast. Recruitment of Ken Sawin, an accomplished researcher working on the fission yeast cytoskeleton, as a meeting organizer helped us to recruit an impressive panel of session chairs including Sir Paul Nurse (Nobel Prize winner for cell cycle studies in fission yeast), representing many fields of cell biology in both yeasts. The resulting meeting had an exciting mix of research from the two systems, which highlighted both deep conservation (e.g., of Tor nutrient signaling, or kinetochore and spindle checkpoint pathways in the two systems) and remarkable differences in the way that the different systems use conserved pathways (e.g., the mechanisms of polarization).

Among this year's many highlights, diverse presentations featured new cutting-edge biophysical approaches using advances in single-particle tracking methodologies to address the mechanisms of septin filament assembly (Amy Gladfelter), endocytosis (Marko Kaksonen), and ultraslow diffusion in yeast plasma membranes (Frans Bianchi). In a technical tour de force, Paul Nurse presented single-molecule DNA combing studies that analyzed enormous (up to 5 MB) DNA molecules, revealing hitherto unappreciated large-scale clustering in the pattern of DNA replication origin firing during S phase. And creative and ingenious approaches were presented to measure rare "escape events" from chromatin silencing (Jasper Rine's lab), to reveal unexpected aspects of the pheromone signaling pathway using periodic stimulation (Tim Elston and Beverly Errede labs), and to map the order of chaperone actions during protein folding (Michael McMurray lab).

Several presentations solved long-standing puzzles. For example, Peter Pryciak revealed the detailed mechanism whereby the mating pathway actually blocks cell cycle progression; Yoshi Watanabe in fission yeast and Sue Biggins in budding yeast revealed the detailed mechanism whereby checkpoint components are recruited to kinetochores. Others identified new cell biological phenomena—for example, mega-autophagy during meiosis and sporulation, from Marc Meneghini's lab, and mother-daughter differences in pH regulation leading to aging, from Dan Gottschling. And yet others discovered new kinds of mutant phenotypes revealing intriguing new puzzles in systems that we thought we understood (e.g., on the role of the nuclear envelope in maintaining the integrity of the microtubule-organizing center). In some areas, different experiments provoked a stimulating clash of models (e.g., on size control in fission yeast, or pheromone gradient tracking in budding yeast). And in all areas, presentations and posters led to enthusiastic questions and discussions.

Although funding constraints and other factors relating to the November timing reduced attendance at this year's meeting, the combined yeasts meeting drew in many researchers who had never attended a Cold Spring Harbor meeting before, and attendees were very enthusiastic about the high level of the science at the meeting as well as its egalitarian format and intimate setting.

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; and Sunrise Scientific.

D. Gottschling, A. Selmecki

PROGRAM

Cytoskeleton and Cytokinesis
Chairpersons: K. Gould, *HHMI/Vanderbilt University, Nashville, Tennessee;* M. Balasubramanian, *Temasek Life Sciences Laboratory, Singapore*

Membranes, Organelles, and Traffic
Chairpersons: S. Lemmon, *University of Miami, Florida;* M. Kaksonen, *EMBL Heidelberg, Germany*

Polarity and Morphogenesis
Chairpersons: A. Gladfelter, *Dartmouth College, Hanover, New Hampshire;* D. Kovar, *University of Chicago, Illinois*

Stress Responses and Signaling
Chairpersons: K. Shiozaki, *Nara Institute of Science & Technology, Japan;* J. Frydman, *Stanford University, California*

Mating and Meiosis
Chairpersons: P. Pryciak, *University of Massachusetts Medical School, Worcester;* J. Thorner, *University of California, Berkeley*

Chromosomes and Mitosis
Chairpersons: S. Biggins, *Fred Hutchinson Cancer Research Center, Seattle, Washington;* Y. Watanabe, *University of Tokyo, Japan*

Cell Cycle Control and Proteolysis
Chairpersons: S. Emr, *Cornell University, Ithaca, New York;* P. Nurse, *Francis Crick Institute, London, United Kingdom*

Nuclear Structure and Function
Chairpersons: T. Davis, *University of Washington, Seattle;* J. Rine, *University of California, Berkeley*

J. Rine, A. Kachroo

P. Nurse, J. Pringle

Precision Medicine: Personal Genomes and Pharmacogenomics

November 13–16 171 Participants

ARRANGED BY **Ann Daly,** Newcastle University Medical School, United Kingdom
Nicholas Katsanis, Duke University
Deanna Kroetz, University of California, San Francisco
Jim Lupski, Baylor College of Medicine

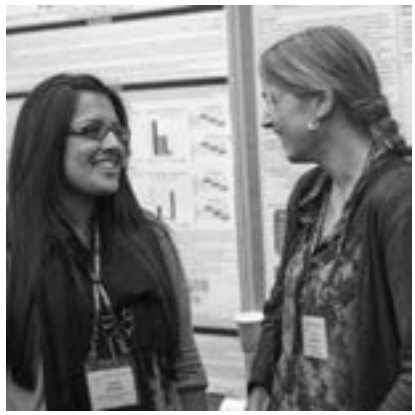
N. Katsanis, A. Daly, D. Kroetz, J. Lupski

This sixth meeting on Personal Genomes again combined both the content of the Personal Genomes Meeting and that of the Pharmacogenomics Meeting to bring together scientific communities interested in gleaning medically actionable information from individual personal genomes. The meeting was titled "Precision Medicine: Personal Genomes and Pharmacogenomics" as a further step towards precision medicine that utilizes genomic information to guide differential diagnoses and potentially optimizing and directing therapeutic interventions based on individual genetic/genomic variation. The meeting was initiated with an outstanding and personal presentation from Hugh Rienhoff, who spoke about his quest to find the underlying genetic susceptibility/contribution(s) to his daughter's phenotype—highlighting the challenges his family faced seeking a diagnosis for a condition not known to exist. Hugh's struggles as a parent were paralleled by his struggles as a scientist and medical geneticist who identifies a novel gene and is faced with the burden of proof for causality. The keynote speaker was both heartening and humbling in that the power of defining disease at the molecular level was apparent, yet our ignorance was further revealed. The keynote address was followed by a terrific Opening Session with a series of talks on genomics to guide differential diagnosis and therapy in which it became obvious that personal genomes are being used around the world at the leading medical institutions by thought leaders in genomics who are trying to leverage the clinical utility of information that can be gleaned from personal genomes. It ended with a powerful description of the United Kingdom 100,000 clinical genomes project. The second session dealt with somatic cell genomics and cancer, and the third on germline genomics emphasizing Mendelizing traits and locus heterogeneity. Three other sessions included genomic variation and common traits/disease, clinical integration of genomic variation with particular reference to recent advances in pharmacogenomics and evolving genomic technologies. An obvious change over last year's meeting was the sheer amount of data and talks that discussed 1000 clinical exomes or 1000 trio analyses of exomes from children with developmental disabilities! A lively and informative ethics panel was presented in which challenges of incidental or secondary findings were discussed in the context of

E. Kiruluta, M. Reese, E. Mardis, M. Singleton

S. Santos, R. Karni

H. Fang, G. Highnam



S. Jhangiani, C. Van Diemen

whether these were something to fear or clinical opportunities to help both physicians and patients contextualize their genetic susceptibilities. Finally, a keynote address was given by Richard Durbin on human genome sequencing from reference to populations to individuals that discussed some of the remarkable progress during this journey. The insights from an individual who has had a large role in leading us down some of the paths were indeed thought provoking. Overall, the meeting was a celebration of the advances of genomic technologies as they transition to personal application, but it also served as a sobering reminder of the magnitude of the task ahead with regard to interpretation and the fundamental, largely unsolved, challenges in distilling clinically actionable items from personal genomes.

This meeting was funded, in part, by the National Institute of General Medical Sciences, a branch of the National Institutes of Health; Life Technologies; Real Time Genomics; and Illumina.

PROGRAM

Genomics to Guide Differential Diagnosis and Therapy
Chairperson: J. Lupski, *Baylor College of Medicine, Houston, Texas*

Keynote Speaker
H.Y. Rienhoff, Jr., *Children's Hospital Oakland Research Institute, San Carlos, California*

Somatic Cell Genomics and Cancer
Chairperson: D. Kroetz, *University of California, San Francisco*

Germline Genomics, Mendelizing Traits, and Locus Heterogeneity
Chairperson: L. Biesecker, *NHGRI, National Institutes of Health, Bethesda, Maryland*

Genomic Variation in Common Traits/Disease
Chairperson: R. Durbin, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Clinical Integration of Genomic Variation
Chairperson: A. Daly, *Newcastle University Medical School, United Kingdom*

Ethics Panel: Incidental Findings or Good Medical Practice?
Moderator: N. Katsanis, *Duke University, Durham, North Carolina*

Evolving Genomic Technologies
Chairperson: R. Gibbs, *Baylor College of Medicine, Houston, Texas*

Keynote Speaker
R. Durbin, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Harnessing Immunity to Prevent and Treat Disease

November 20–23 107 Participants

ARRANGED BY **Susan Kaech,** HHMI/Yale University
Robert Seder, Vaccine Research Center, NIAID, National Institutes of Health
Susan Swain, University of Massachusetts Medical School

Participants at this broad-based meeting explored the design of novel vaccines, the immune response, and delivery systems. Three keynote addresses were delivered by internationally renowned scientists Michel Nussenzweig, Rafi Ahmed, and Antonio Lanzavecchia.

The focal points of the scientific sessions are listed below. There were 32 talks and 41 posters of largely unpublished work presented on these topics, with a significant amount of lively discussion. The 2013 conference was the last of a long series of meetings. The participating scientists in this field look forward to the incorporation of these issues into related CSHL meetings in the future.

This meeting was funded, in part, by the National Institute of Allergy and Infectious Diseases, a branch of the National Institutes of Health.

PROGRAM

Keynote Address: Harnessing Immunity to Prevent and Treat HIV Infection
M. Nussenzweig, *HHMI/The Rockefeller University*

Innate Programming of Adaptive Immunity
Chairperson: K. Fitzgerald, *University of Massachusetts Medical School, Worcester*

Organ-Specific Immunity I
Chairperson: S. Kaech, *HHMI/Yale University, New Haven, Connecticut*

Keynote Address: Human Immune Memory to Vaccination
R. Ahmed, *Emory University School of Medicine*

Transcriptional and Metabolic Control of Adaptive Immunity
Chairperson: E.J. Wherry, *University of Pennsylvania School of Medicine, Philadelphia*

Organ-Specific Immunity II
Chairperson: Y. Belkaid, *NIAID, National Institutes of Health, Bethesda, Maryland*

R. Lier, S. Kaech

S. Nedospasov, L. Delamarre

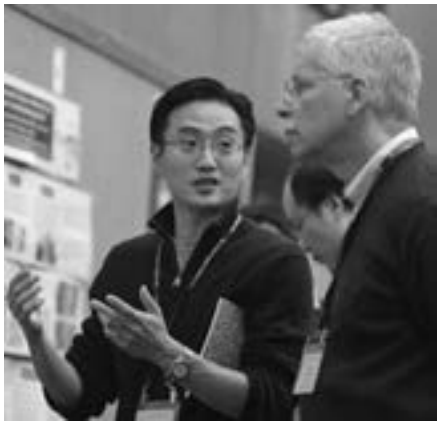
The Impact of Age on Immunity
Chairperson: J. Goronzy, *Stanford University School of Medicine, California*

Keynote Address: Analytic Vaccinology
A. Lanzavecchia, *Institute for Research in Biomedicine, Switzerland*

The Immunological Ascent of Man
Chairperson: R. Seder, *Vaccine Research Center, NIAID, National Institutes of Health, Bethesda, Maryland*

K. Vora, R. Ahmed

K. Yadava



J. Yu, A. Lanzavecchia

Plant Genomes and Biotechnology: From Genes to Networks

December 4–7 124 Participants

ARRANGED BY **Mary Lou Guerinot,** Dartmouth College
Todd Mockler, Donald Danforth Plants Science Center
Detlef Weigel, Max-Planck Institute for Developmental Biology

T. Mockler, D. Weigel, M.L. Guerinot

This eighth Cold Spring Harbor Laboratory conference continued to demonstrate a robust attendance, both from the United States and from abroad. It attracted more than 120 registrants, presenting over 94 abstracts, offering a snapshot of the latest developments in the fields of plant genomics, biotechnology, and systems biology. There were 35 invited talks and one keynote address. The remaining 59 talks were all selected for presentation by the organizers from openly submitted abstracts.

This year, abstracts covered a wide variety of topics in modern plant biology, including gene regulatory networks, in vivo imaging technologies, plant genome analysis, genome assembly, epigenetics/epigenomics, natural variation, diurnal and circadian rhythms, synthetic pathway engineering, plant hormone signaling, and developmental regulation. There was also an emphasis on emerging high-throughput controlled-environment and field-based phenotyping systems complementary to parallel developments in 'omics technologies that together enable genotype–phenotype associations. There were also talks of broad interest to the plant science community, including presentations on the Bill & Melinda Gates Foundation's investments in agricultural research and development and the iPlant Collaborative's unified cyberinfrastructure for plant science. The sessions in the conference are listed below.

The keynote address was delivered by Dr. Joseph Ecker, who discussed the layering of multiple levels of 'omics assays in *Arabidopsis* to understand the system underlying gene regulation and the interactions of gene network components with the genome itself. Dr. Ecker's talk emphasized a number of technologies for whole-genome analysis in *Arabidopsis* that were pioneered in his lab and emphasized the need for a holistic view involving multiple 'omics approaches coupled with

D. Ware, G. Giuliano

J. Nemhauser, R. Simon

genetics to understand the plant cell as a system. This talk was extremely well received and generated an active follow-up discussion.

This meeting was also the first CSHL Plant Genomes and Biotechnology conference to be completely open on Twitter (using hashtag #cshlplant13).

PROGRAM

New Frontiers
Chairperson: D. Weigel, *Max-Planck Institute for Developmental Biology, Tübingen, Germany*

Synthetic Biology and Networks
Chairperson: I. Lee, *Yonsei University, Seoul, Korea*

Abiotic and Biotic Signals
Chairperson: M.L. Guerinot, *Dartmouth College, Hanover, New Hampshire*

Epigenetics
Chairperson: J. Parker, *Max-Planck Institute for Plant Breeding Research, Cologne, Germany*

Biodiversity and Adaptation
Chairperson: L. Hererra-Estrella, *National Polytechnic Institute, Irapuato, Mexico*

Hormones and Development
Chairperson: S. Mackenzie, *University of Nebraska, Lincoln*

Keynote Speaker
J. Ecker, *HHMI/The Salk Institute, San Diego, California*

Genotype–Phenotype Map
Chairperson: J. Nemhauser, *University of Washington, Seattle*

Rat Genomics and Models

December 11–14 133 Participants

ARRANGED BY **Edwin Cuppen,** Hubrecht Institute, The Netherlands
Aron Geurts, Medical College of Wisconsin
Michael Gould, University of Wisconsin, Madison
Bina Joe, University of Toledo College of Medicine

This eighth winter biotechnology conference has been held biannually since 1999 at Cold Spring Harbor Laboratory and 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 the alternating years, with the most recent meetings being held in Cambridge, United Kingdom (2012), Kyoto, Japan (2010), Hinxton, United Kingdom (2008), and Melbourne, Australia (2006). The primary goals of this meeting were to (1) promote interactions among biomedical researchers who use rat models in the study of emerging technology, genomics, physiology, pathophysiology, metabolism, neuroscience, and oncology; (2) provide young investigators and trainees an opportunity to network with leaders in the rat genomics community; and (3) provide an interface between the research community and the entities that support biomedical research in which rat models are used.

The meeting was organized into six sessions featuring both invited speakers and those selected from submitted abstracts. An opening keynote lecture featured Dr. Ross Hardison, who described his ongoing interest in the rat as a means to understand the impact of genomics and epigenetic analysis to dissect out causative variants and uncover mechanistic insights into physiology and pathology.

Chairpersons and sessions are listed below. Invited speakers featured for each session were Dr. Tomoji Mashimo (Session 1); Drs. Timothy Aitman, Paul Flicek, and Norbert Hübner (Session 2); Drs. James Shull and David Samuelson (Session 3); Drs. Percio Gulko, Steve Britton, and

E. Cuppen, A. Geurts, B. Joe, M. Gould

Elizabeth Blankenhorn (Session 4); Drs. Mingyu Liang and Ted Kurtz (Session 5); and Drs. Boris Tabakoff and Fabio Cruz (Session 6).

The closing keynote address featured Dr. Howard Jacob, who was the original founder of the conference series and longtime champion of rat genomics. He discussed strategies for the rat community to move successfully and collaboratively forward in a difficult funding environment by capitalizing on human genetics and the excellent physiological and disease models that the rat community has developed. In sum, 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

Keynote Speaker
R. Hardison, *Pennsylvania State University, State College*

Emerging Technologies: Stem Cells and Genome Manipulation
Chairperson: A. Geurts, *Medical College of Wisconsin, Milwaukee*

Genomics, Epigenetics, and Integrative Biology
Chairperson: E. Cuppen, *Hubrecht Institute, Utrecht, The Netherlands*

Tumor Biology
Chairperson: M. Gould, *University of Wisconsin, Madison*

Metabolism, Inflammation, and Exercise Physiology
Chairperson: A. Kwitek, *University of Iowa, Iowa City*

Cardiovascular Diseases
Chairperson: B. Joe, *University of Toledo College of Medicine, Ohio*

Neurobiology, Behavior, and Addiction
Chairperson: A. Geurts, *Medical College of Wisconsin, Milwaukee*

Keynote Speaker
H. Jacob, *Medical College of Wisconsin, Milwaukee*

H. Jacob, N. Hubner

O. Hummel, C. Mieth, K. Saar

P. Gellar

POSTGRADUATE COURSES

Workshop on Leadership in Bioscience

February 22–25

INSTRUCTORS **C.M. Cohen,** Science Management Associates
D. Kennedy, Workshop Cofacilitator

This was a highly interactive 3.5-day workshop in which participants developed the skills necessary to lead and interact effectively with others, in both one-on-one and group settings. The workshop focused on techniques, situations, and challenges that relate specifically to leading and managing in the scientific workplace. It emphasized learning by doing and involved role playing, giving and receiving feedback, and group problem solving. Much of the learning was peer-to-peer. Participants were expected to discuss their own experiences and listen to others as they discuss theirs. The workshop helped participants identify areas where they need guidance and growth, as well as how to capitalize on areas of strength. Participants had the opportunity to share their experiences and challenges with others and to receive feedback and guidance from others with experience in leading scientists in a variety of settings. At the end of the course, participants were linked through a unique on-line community in which they continued learning from one another and from the course instructors.

This course was supported with generous support from American Express Philanthropy.

PARTICIPANTS

Ahmari, S., Ph.D., Columbia University, New York
Carter, M., Ph.D., Center for Disease Control and Prevention, Atlanta, Georgia
Chen, P., Ph.D., Kessler Foundation, West Orange, New Jersey
Crowder, R., Ph.D., Penn State, Milton S. Hershey Medical Center, Hershey, Pennsylvania
Fantana, A., Ph.D., Bowling Green State University, Ohio
Gutteling, E., Ph.D., Rijik Zwaan Breeding BV, The Netherlands
Hamerlik, P., M.D., Danish Cancer Society Research Center, Denmark
Hanlon, C., Ph.D., Medical University of South Carolina, Charleston
Kain, J., Ph.D., Harvard University, Cambridge, Massachusetts
Karst, A., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Krause, S., Ph.D., Boston Children's Hospital, Boston, Massachusetts
Kvaskoff, M., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Lee, J., Ph.D., ArQule, Woburn, Massachusetts
Marie, C., Ph.D., University of Virginia, Charlottesville
McKay, S., M.S., University of Vermont, Burlington
Norholm, M., Ph.D., Technical University of Denmark
Noustos, C., Ph.D., Cold Spring Harbor Laboratory
Ray, R., Ph.D., Baylor College of Medicine, Houston, Texas
Reis, T., Ph.D., University of Colorado Medical School, Aurora
Schweizer B.A., Ph.D., University of Pennsylvania, Philadelphia
Seib, P., Ph.D., University of Strathclyde, Glasgow, Germany
Timmes, A., B.S., Rocky Mountain Laboratories, Hamilton, Montana
Tseng, K., Ph.D., University of Nevada, Las Vegas
Ulrich, T., Ph.D., Massachusetts Institute of Technology, Cambridge
Wang, Kai, Ph.D., University of Southern California, Los Angeles
White, E., Ph.D., Harvard Medical School, Cambridge, Massachusetts

SEMINAR

Miller, K. Brown University, Providence, Rhode Island: Science in the public eye.

Protein Purification and Characterization

April 3–16

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
S. Callaci, University of Wisconsin, Madison
M. Donovan, Brandeis University, Waltham, Massachusetts
P. Kwong, University of California, Los Angeles
Y.-C. Lee, M.D. Anderson Cancer Center/University of Texas, Houston
M. Spellberg, Brandeis University, Waltham, Massachusetts
N. Thompson, University of Wisconsin, Madison
W. Turki-Judeh, University of California, Los Angeles

This course was for scientists who were unfamiliar with techniques of protein isolation and characterization. The course was extremely rigorous and included laboratory work during the days, as well as lectures with discussions and student talks in the evenings. Students are typically graduate students, postdoctoral scholars, staff scientists, and professors with specialized scientific expertise who now need to learn about protein purification and characterization. The course emphasized laboratory strategies and current best practices in the field.

Each student in the course became familiar with major techniques in protein purification by performing four separate isolations: (1) a regulatory protein (calmodulin) from muscle tissue (chicken gizzards), (2) a sequence-specific DNA-binding protein (transcription factor AP1) from HeLa cell nuclei, (3) a recombinant protein overexpressed as inclusion bodies in *E. coli*, and (4) a membrane-bound protein (insulin receptor) from rat liver.

Students were divided into four groups of four and spent three days in each of the above four modules. In addition to the primary purification in a given module, a number of relevant characterizations were performed on each protein, giving students experience with immunological and biochemical assays, peptide mapping, protein sequencing, and mass spectrometry. Students learned bulk fractionation and electrophoretic and chromatographic techniques, including 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. Students also learned procedures for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Evenings in the course involved lectures about topics that complement the laboratory curriculum: protein structure; modification of proteins; methodologies for protein purification, stabilization, and characterization; and applications of protein biochemistry to molecular cell biology and cancer research.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Barrio, G.C., B.S., Gene Center, Munich, Germany
Basu, J., Ph.D., Lebanon Hospital Center, Bronx, New York
Chuang, C., Ph.D., M.D. Anderson Cancer Center, Houston, Texas
Fredens, J., M.S., University of Southern Denmark, Odense
Gomes, A., M.S., University of Copenhagen, Denmark
Jay, S., Ph.D., Brigham and Women's Hospital, Cambridge, Massachusetts
Johnson, M., B.S., Texas A&M University, College Station
Manna, J., B.A., Vanderbilt University Medical Center, Nashville, Tennessee
McCoy, F., B.Sc., St. Jude Children's Research Hospital, Memphis, Tennessee
Nelson, J., M.S., University of Amsterdam, The Netherlands
Quijada, J., B.S., Northeastern University, Boston, Massachusetts
Sami, F., Ph.D., Howard University, Washington, DC
Tsai, Y.C., Ph.D., National Cancer Institute, Frederick, Maryland
Wall, E., B.S., Virginia Commonwealth University, Richmond
Wylie, C.S., Ph.D., Brown University, Providence, Rhode Island
Yi, J. (Eva), Ph.D., University of Oklahoma, Norman

SEMINARS

Burgess, R., University of Wisconsin, Madison: Introduction to protein purification. Doing protein purification in the mid-60s and 1-second purification today.
Courey, A., University of California, Los Angeles: System-wide analyses of Groucho and SUMO in *Drosophila.*
Lazic, A., Nano Temper Technologies, San Francisco, California: Some like it hot: Biomolecule analytics using microscale, thermophoresis MST.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Secretome analysis of prostate cancer bone metastasis.
Marr, M., Brandeis University, Boston, Massachusetts: Multilevel control of gene expression in response to cellular stress.
Pappin, D., Cold Spring Harbor Laboratory: Introduction to mass spectrometry of proteins.
Thompson, N., University of Wisconsin, Madison: Monoclonal antibodies and gentle immunoaffinity chromatography.

Quantitative Imaging: From Cells to Molecules

April 3–16

INSTRUCTORS	**J. Waters,** Harvard Medical School, Boston, Massachusetts **T. Wittmann,** University of California, San Francisco
CO-INSTRUCTORS	**L. Cameron,** Dana-Farber Cancer Institute, Boston, Massachusetts **B. Huang,** University of California, San Francisco **M. Krummel,** University of California, San Francisco
ASSISTANTS	**R. Citron,** University of California, San Francisco **K. Corbin,** University of California, San Francisco **A. Ettinger,** University of California, San Francisco **T. Lambert,** Washington State University, Pullman **H. Pemble,** 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 using 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), super-resolution 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. They used the techniques to address specific quantitative questions and then discussed the results as a group, learning to troubleshoot the common problems that occurred 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 ratiometric "biosensors" (including FRET), and super-resolution techniques. Students also learned specimen preparation for microscopy, including fixation and immunofluorescence in tissue culture cells, choosing fluorescent proteins, working with live samples requiring environmental control, and more.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Collu, G., Mount Sinai School of Medicine, New York
Das, A., Ph.D., National Heart, Lung, and Blood Institute, Bethesda, Maryland
Durham, N., B.Sc., Mount Sinai School of Medicine, New York
Evans, D., B.S., Mayo Clinic, Rochester, Minnesota
Githaka, J., B.Sc., University of Alberta, Edmonton, Canada
Ionescu, I., Ph.D., Max-Planck Institute of Psychiatry, Munich, Germany
Magadan, J., Ph.D., National Institutes of Health, Bethesda, Maryland
Montero, L., Paula, Ph.D., Harvard Medical School, Boston, Massachusetts
Pease, S., B.S., Harvard University, Cambridge, Massachusetts
Peters, N., Ph.D., Harvard Medical School, Boston, Massachusetts
Rino, J., Ph.D., Instituto de Medicina Molecular, Lisbon, Portugal
Salter, A., B.Sc., University of Manchester, United Kingdom
Spelke, D., B.S., University of California, Berkeley
Titov, D., Ph.D., Massachusetts General Hospital, Boston
Tyler, J., B.S., University of North Carolina, Chapel Hill

SEMINARS

Betzig, E., Janelia Farm Research Campus, Ashburn, Virginia: Improving the spatiotemporal resolution of fluorescence microscopy.
Cameron, L., Dana-Farber Cancer Institute, Boston, Massachusetts: Confocal microscopy.
Huang, B., University of California, San Francisco: Super-resolution microscopy: STORM/PALM. Super-resolution microscopy: Structured illumination. Life inside the cell.
Goodwin, P.l., Applied Precision, Inc., Issaquah, Washington: Deconvolution.
Krummel, M., University of California, San Francisco: TIRF. Principles, benefits, and application of multiphoton microscopy. Live imaging of immune cell dynamics in viable tissues.
Patterson, G., National Institutes of Health, Bethesda, Maryland: Fluorescent proteins.
Waters, J., Harvard Medical School, Boston, Massachusetts: Quantitative microscopy basics. Objective lenses. Transmitted light microscopy. Digital cameras. Quantitative digital imaging. Ratio imaging and FRET.
Wittmann, T., University of California, San Francisco: Live-cell imaging. Live confocal, FRAP, and photoactivation. CLASP-mediated local exocytosis facilitates extracellular matrix degradation and focal adhesion turnover. Sample preparation for fluorescence microscopy. Fluorescence microscopy.

Cell and Developmental Biology of *Xenopus*

April 5–16

INSTRUCTORS **A. Sater,** University of Houston, Texas
G. Thomsen, Stony Brook University, New York

ASSISTANTS **T. Nakayama,** University of Virginia, Charlottesville
J. Oomen-Halagos, Stony Brook University, New York
K. Pfister, University of Virginia, Charlottesville

Xenopus is the leading vertebrate model for the analysis 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. Moreover, recent advances in *Xenopus* genomics offer new opportunities to integrate computational strategies with experimental approaches. The course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *Xenopus* development. An important element was the informal interaction between students and course faculty.

Technologies covered included oocyte and embryo culture, lineage analysis and experimental manipulation of embryos, time-lapse imaging of morphogenesis, gain- and loss-of-function analysis using mRNAs and antisense oligonucleotides, whole-mount in situ hybridization, immunocytochemistry, genomics and bioinformatics, chromatin immunoprecipitation, 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.

PARTICIPANTS

Barber, A., Ph.D., The University of East Anglia, Norwich, United Kingdom
Chowanadisai, W., Ph.D., University of California, Davis
De Domenico, E., Ph.D., MRC-National Institute for Medical Research, London, United Kingdom
Jevtic, P., B.S., University of Wyoming, Laramie
Kwon, T., Ph.D., Center for Systems and Synthetic Biology, Austin, Texas
O'Connell, L., Ph.D., Harvard University, Cambridge, Massachusetts
Petridou, N., B.Sc., University of Cyprus, Nicosia, Cyprus
Pickett, M., B.S., North Carolina State University, Raleigh
Reis, R.A., M.S., Federal University of Rio de Janeiro, Brazil
Reyes, C., B.S., University of Michigan, Ann Arbor
Schuh-Herta, S., Ph.D., Stanford University, Palo Alto, California
Shah, V., B.S., University of Houston, Texas
Smith, H., M.S., University of Texas, Austin
Speer, K., B.A., Perelman School of Medicine, Philadelphia, Pennsylvania
Tisler, M., Diplom., University of Hohenheim, Stuttgart, Germany
Zhu, K., B.S., Leiden University, The Netherlands

SEMINARS

El-Hodiri, H., Ohio State University, Columbus; The retinal homeobox (*Rx*) gene plays essential roles in the regenerating retina.
Gilchrist, M., National Institute for Medical Research, London, United Kingdom: Using high-resolution RNA-Seq data to untangle mechanisms of gene activation in early development.
Grainger, R., University of Virginia, Charlottesville: Analysis of eye formation in *Xenopus:* Model system for genetic and genomic studies of determination, induction, and organogenesis.
Hannon, G., Cold Spring Harbor Laboratory: A small RNA-based immune system defends the germline against mobile elements.
Harland, R., University of California, Berkeley: Spemann's organizer.
Keller, R., University of Virginia, Charlottesville: Gastrulation and morphogenesis.
Khokha, M., Yale University, New Haven, Connecticut: Modeling congenital heart disease genes in *Xenopus*.
Klein, P., University of Pennsylvania School of Medicine, Philadelphia: Mechanisms of Wnt signaling in early development.
Nakayama, T., University of Virginia, Charlottesville: Transgenic approaches for manipulating gene expression in *Xenopus.*
Peshkin, L., Harvard Medical School, Boston, Massachusetts: Quantitative molecular embryology: Coupled proteomic-transcriptomic analysis reveals developmental strategies.
Shindo, A., HHMI/University of Texas, Austin: In vivo imaging of cellular and subcellular behaviors during morphogenesis.

Workshop on Autism Spectrum Disorders

June 5–11

INSTRUCTORS **D. Geschwind,** University of California Center for Autism Research, Los Angeles
P. Levitt, University of Southern California, Los Angeles
S. Spence, Children's Hospital Boston/Harvard University, Massachusetts

ASSISTANT **N. Parikshak,** University of California, Los Angeles

Autism spectrum disorders (ASD) are developmental disorders with complex phenotypes defined by a triad of symptoms that include disrupted social abilities, verbal and nonverbal communication skills, and restricted interests with repetitive behaviors. Co-occurring neurological and medical conditions are common in the disorder. The underlying etiology remains a mystery, but ASD is one of the most highly heritable of neuropsychiatric disorders. This workshop examined the dimensions of ASD on various levels, which included 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, this course explored and debated controversial topics and challenges to basic assumptions in the field. An exceptional faculty with diverse interests brought the most up-to-date results and theories to the students, making this workshop a valuable resource for young researchers starting out in this fast-moving and expansive field. Not only did it help them build the foundation for their future research, it 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. Importantly, students also had free time for reading, informal discussions, and recreation on the beautiful Banbury Center campus.

This course was supported with funds provided by the Nancy Lurie Marks Family Foundation.

PARTICIPANTS

Albers Prock, L., M.D., Boston Children's Hospital, Boston, Massachusetts
Barak, B., Ph.D., Massachusetts Institute of Technology, Cambridge
Boin Choi, A., M.Ed., Harvard Graduate School of Education, Cambridge, Massachusetts
Brimberg, L., Ph.D., Feinstein Institute for Medical Research, Manhasset, New York
Brumback, A., M.D., Ph.D., University of California, San Francisco
Burrows, E., Ph.D., Florey Institute of Neuroscience and Mental Health, Parkville, Victoria, Australia
Karhson, D., Ph.D., Tulane University, New Orleans, Louisiana
Kim, S., Ph.D., Stanford University, California
Kouser, M., Ph.D., University of Texas Southwestern Medical Center, Dallas
Levin, A., M.D., Boston Children's Hospital, Boston, Massachusetts
Makhlouf, M., Ph.D., Diderot University, Paris, France
Miodovnik, A., M.D., Boston Children's Hospital, Massachusetts
Morin Duchesne, X., M.S., Université de Montréal, Montreal, QC, Canada
Sidorov, M., Ph.D., Massachusetts Institute of Technology, Cambridge
Speed, H., Ph.D., University of Texas Southwestern Medical Center, Dallas
Swarup, V., Ph.D., University of California, Los Angeles
Yau, S., Ph.D., ARC Centre of Excellence in Cognition and Its Disorders, Sydney, Australia
Zermeño, A., Ph.D., Universidad de Salamanca, Spain

SEMINARS

Akbarian, S., Mount Sinai School of Medicine, New York: Epigenetics.
Bird, G., King's College London, United Kingdom: Mirror neurons, theory of mind, and social behavior.
Fombonne, E., Oregon Health and Science University, Portland: Epidemiology of ASD.
Geshwind, D., University of California, Los Angeles: Introduction to human genetics and genomics/gene expression findings in ASD.
Geschwind, D., University of California, Los Angeles; Levitt, P., University of Southern California; Spence, S., Boston Children's Hospital, Massachusetts; Introduction to the course and student assignments.
Hensch, T., Harvard University, Boston, Massachusetts: Clinical period plasticity.
Iversen, P., Co-Founder of Cure Autism Now: Autism close up and personal.
Kasari, C., University of California, Los Angeles: Engaging autism: Active ingredients for early interventions. Social skills: Methods, models, and mediating agents.
Levitt, P., University of Southern California, Los Angeles: Basics of neural development, cellular and molecular neuropathology, inflammation.
Nelson, C., Boston Children's Hospital, Massachusetts: Electrophysiological imaging methods in ASD. Early identification in ASD.
Pasca, S., Stanford University, California: Cellular models for ASD in humans.
Pelphrey, K., Yale University, New Haven, Connecticut: Searching for neuroendophenotypes of autism.
Powell, C., University of Texas, Austin: Interpreting animal models of ASD.
Rapin, I., Albert Einstein School of Medicine, Bronx, New York: Autism2013: 60 Years of personal experience.
Spence, S., Boston Children's Hospital, Massachusetts: Informal discussion of clinical assessments. Medical comorbidities.
Stevens, B., Harvard Medical School, Boston, Massachusetts: Synaptic physiology.
Stone, W., University of Washington, Seattle: Clinical presentation and phenotypic variation. An SD phenotypic variability outcome.
Suomi, S., NICHD, National Institutes of Health, Bethesda, Maryland: Evolutionary perspectives on social behavior.
Tsien, R., New York University, New York: Circuits and functional physiology: Oxytocin.
Veenstra-Vander Weele, J., Vanderbilt University, Nashville, Tennessee: From mouse to man: The Path to new treatments in ASD.
Weisskopf, M., Harvard School of Public Health, Longwood, Massachusetts: Environmental contributions.
Young, L., Emory University, Atlanta, Georgia: Oxytocin and social cognition: Implications for novel therapies for autism.

Single-Cell Analysis

June 5–18

INSTRUCTORS
J. Eberwine, University of Pennsylvania Perelman School of Medicine, Philadelphia
C. McMurray, University of California, Berkeley/Lawrence Berkeley Laboratory

ASSISTANTS
T. Ball, NDRI Resource, Philadelphia, Pennsylvania
P. Buckley, Janssen Research & Development, Spring House, Pennsylvania
H. Budworth, Lawrence Berkeley National Laboratory, Berkeley, California
C. Canaria, Lawrence Berkeley National Laboratory, Berkeley, California
K. Frankel, Lawrence Berkeley National Laboratory, Berkeley, California
D.-Y. Lee, Lawrence Berkeley Laboratory, Seoul, Korea
J. Morris, University of Pennsylvania School of Medicine, Philadelphia

The goal of this new 2-week course was to familiarize students with the most recent cutting-edge technologies for characterization of cells. Important in this process was highlighting the advantages to analysis of single cells in isolation and in their natural microenvironment. Sections of the course were taught by scientists who are experts in particular areas of single-cell analysis. Topics that were covered included quantitative single-cell analysis by RNA-Seq, genomic DNA analysis, epigenetics, RNA localization analysis, proteomics, protein translation, and metabolomics. The techniques that were taught included real-time live cell quantification where appropriate. Multiple techniques to isolate specific cell populations and individual cells were also taught. The course included the use of model systems such as mouse, *Drosophila*, *C. elegans*, *Aplysia*, and *Planaria*.

This course was generously supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Brosnahan, M., Ph.D., Woods Hole Oceanographic Institution, Massachusetts
Carre, G.-A., Ph.D., MRC, Harwell, United Kingdom
Gini, B., Ph.D., University of California, San Diego, La Jolla
Guibentif, C., B.Sc., Lund University, Sweden
Han, M.-E., Ph.D., Pusan National University, Yangsan, South Korea
Hill, C., Ph.D., Burke-Cornell Medical Research Institute, White Plains, New York
Jouhilahti, E.-M., Ph.D., Karolinska Institutet, Huddinge, Sweden
Kolesnikova, A., M.S., Institute of Cell Biophysics, Pushchino, Russia
Kudryavtseva, A., Ph.D., Engelhardt Institute of Molecular Biology RAS, Moscow, Russia
Lundin, E., M.S., Stockholm University, Sweden
Madsen, J., B.Sc., University of Southern Denmark, Odense
Mande, K., Ph.D., King Abdullah University of Science & Technology, Jeddah, Saudi Arabia
Oka, Y., B.S., Nara Institute of Science and Technology, Ikoma-shi, Japan
Proctor, A., Ph.D., University of North Carolina, Chapel Hill
Roberts, K., Ph.D., St. Jude Children's Research Hospital, Memphis, Tennessee
Shakur, R., M.D., University of Cambridge, United Kingdom
Vermehren-Schmaedick, A., Ph.D., Oregon Health and Science University, Portland

SEMINARS

Albritton, N., University of North Carolina, Chapel Hill: Separating the one from the many.
Botvinick, E., University of California, Irvine: 3-D durataxis: Laser microbeams and mechanobiology.
Eberwine, J., University of Pennsylvania, Philadelphia: The secret lives of single cells.
Kim, J., University of Pennsylvania, Philadelphia: The single-cell RNA landscape and its computational analysis.
Larabell, C., Lawrence Berkeley Laboratory, California: Nanoscale CT scans of single cells.
McMurray, C., Lawrence Berkeley Laboratory, California: Cell type alterations in the brains of Huntington's disease mice.
Montell, D., University of California, Santa Barbara: Life, death, and resurrection at the cellular level.
Roberts, R., University of Southern California, Los Angeles: Using mRNA display to design intrabodies.

Advanced Bacterial Genetics

June 5–25

INSTRUCTORS
D. Hughes, Uppsala University, Sweden
B. Lazazzera, University of California, Los Angeles
F. Yildiz, University of California, Santa Cruz

ASSISTANTS
G. Brandis, Uppsala University, Sweden
N. Howitz, University of California, Los Angeles
L. Townsley, University of California, Santa Cruz

This course presented logic and methods used in the genetic dissection of complex biological processes in diverse bacteria. Laboratory methods included classical mutagenesis using transposons, and mutator strains, 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 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 bacteria (including *E. 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.

The course admitted 18 students, both foreign and US residents, from diverse backgrounds and career levels for intensive (but fun) instruction in microbial genetics.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Angst, D., M.S., ETH Zurich, Switzerland
Barnes, S., B.S., California Institute of Technology, Pasadena
Daughtry, K., Ph.D., National Institute of Environmental Health Science, Research Triangle Park, North Carolina
Goemans, C., M.S., De Duve Institute, Belgium
Hernandez, C., Ph.D., Cornell University, Ithaca, New York
Holwerda, E., M.S., Dartmouth College, Hanover, New Hampshire
Lang, K., B.S., University of Minnesota, St. Paul
Los, J., Ph.D., University of Gdansk, Poland
Murray, S., Ph.D., John Innes Centre, United Kingdom
Noobakhsh, J., B.S., Boston University, Massachusetts
Simari, R., B.S., The Ohio State University, Columbus
Rocio Suarez-Moreno, Z., Ph.D., Instituto de Biotechnologia-Universidad Nacional, Colombia
Stokes, E., B.S., City College, City University of New York
Sysoeva, T., Ph.D., Harvard University, Cambridge, Massachusetts
Tran, C., M.D., University of California, San Francisco
Uehling, J., M.S., Duke University, Durham, North Carolina
Ursell, T., Ph.D., Stanford University, California
Zampieri, M., Ph.D., ETH Zurich, Switzerland

SEMINARS

Darwin, H., New York University, New York: Using genetics to understand mycobacterium tuberculosis pathogenesis.
Grossman, A., Massachusetts Institute of Technology, Cambridge: Horizontal gene transfer: Functions of integrative and conjugative elements.
Kirby, J., University of Iowa, Iowa City: Chemosensory regulation of multicellularity in bacteria.
Levin, B., Emory University, Atlanta, Georgia: Modeling the population and evolutionary dynamics of bacteria for people who prefer to hum equations than solve them. The population biology and evolution of adaptive immunity in bacterial, CRISPR, and too much fun.
Lovett, S., Brandeis University, Waltham, Massachusetts: Replication during starvation: The stringent response.
Schmid, M., Tech Coast Angels, Claremont, California: Use of bacterial genetics in the drug discovery process.
Schweizer, H., Colorado State University, Fort Collins: The mini-Tn7 systems for gram-negative bacteria: A versatile and user-friendly genetic toolbox.
Silhavy, T., Princeton University, New Jersey: Divide and conquer: Genetic dissection of the β-barrel assembly machine.
Snitkin, E., National Institutes of Health, Bethesda, Maryland: Overview of bioinformatics tools for bacterial genetics.

Mouse Development, Stem Cells, and Cancer

June 5–25

INSTRUCTORS	**R. Johnson,** M.D. Anderson Cancer Center, Houston, Texas **X. Sun,** University of Wisconsin, Madison
CO-INSTRUCTORS	**M. Lewandoski,** National Cancer Institute, Frederick, Maryland **D. Wellik,** University of Michigan Medical Center, Ann Arbor
ASSISTANTS	**H. Adams,** M.D. Anderson Cancer Center, Houston, Texas **K. Branchfield,** University of Wisconsin, Madison **Y. Furuta,** RIKEN, Center for Developmental Biology, Kobe, Japan **R. Kadzik,** University of Pennsylvania, Perleman School of Medicine, Philadelphia **B. Larsen,** University of Michigan Medical Center, Ann Arbor **R. Poche,** Baylor College of Medicine, Houston, Texas **N. Schrode**, Memorial Sloan-Kettering Cancer Institute, New York

This intensive laboratory and lecture course was designed for biologists interested in using mouse models to study mammalian development, stem cells, and cancer. Lectures provided the conceptual basis for contemporary research in embryogenesis, organogenesis, embryonic, adult and induced pluripotent stem cells, and cancer biology. Laboratory practicals provided an extensive hands-on introduction to engineering of mouse models, phenotyping, and stem cell technologies. Experimental techniques covered included isolation, in vitro culture and manipulation of pre- and post-implantation embryos, embryo transfer, genetic manipulation of embryonic stem cells, production of chimeras by embryo aggregation and by embryonic stem cell injection, and transgenesis by pronuclear microinjection. The course also introduced the generation and differentiation of embryonic stem cells as well as induced pluripotent stem cells, isolation of mouse embryonic fibroblasts,

focus formation assay, teratoma formation assay, time-lapse microscopy of early embryos, and organ cultures. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including vibratome and cryosectioning, in situ hybridization, immunostaining, skeletal preparation, tissue recombination, and confocal and ultrasound imaging.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Alberton, P., Ph.D., Ludwig-Maximilians University, Munich, Germany
Dominguez, M.I., Ph.D., Boston University School of Medicine, Massachusetts
Fontanet, P., B.S., Institute of Cellular Biology and Neuroscience, Argentina
Funk, M., M.S., University Medical Center Freiburg, Germany
Gama, V., Ph.D., University of North Carolina, Chapel Hill
Garces, J., B.A., University of Pennsylvania, Philadelphia
Han, L., B.S., University of Cincinnati, Ohio
Koutelou, E., Ph.D., University of Texas M.D. Anderson Cancer Center, Smithville
Rooney, T., B.S., University of Massachusetts Medical School, Worcester
Sahai-Hernandez, P., B.S., University of California, San Francisco
Wainwright, E., B.S., University of Queensland, Brisbane, Australia
Wu, P.-H., Ph.D., HHMI/University of Massachusetts, Worcester
Zhang, C., Ph.D., Columbia University, New York
Zong, C., Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Behringer, R., M.D. Anderson Cancer Center, Houston, Texas: Mouse transgenics and reproduction.
Capecchi, M., University of Utah School of Medicine, Salt Lake City: Homologous recombination and knockout mice.
Cleaver, O., University of Texas Southwestern Medical Center, Dallas: Mouse pancreas development and disease. Mouse blood vessel development.
DePinho, R., M.D. Anderson Cancer Center, Houston, Texas: GEMs in cancer and aging.
Egeblad, M., Cold Spring Harbor Laboratory: Live imaging in mouse models of cancer.
Johnson, R., M.D. Anderson Cancer Center, Houston, Texas: Mouse liver development and cancer.
Li, L., Stowers Institute for Medical Research, Kansas City, Missouri: Mouse hematopoiesis.
Lewandoski, M., National Cancer Institute, Frederick, Maryland: Mouse conditional knockout technologies. Mouse somitogenesis.
Looney, M., University of California, San Francisco: Lung imaging in mice.
Lovell-Badge, R., MRC National Institute for Medical, United Kingdom: Sex determination.
Lowe, S., Memorial Sloan-Kettering Cancer Center, New York: Probing cancer drivers and dependencies using mouse models and RNAi.
Morrisey, E., University of Pennsylvania, Philadelphia: Mouse heart development and diseases.
Rivera, J., University of Massachusetts Medical School, Worcester: Mouse postimplantation.
Rossant, J., The Hospital for Sick Children, Toronto, Ontario, Canada: Early mouse embryonic and extraembryonic cell lineage.
Solter, D., Institute of Medical Biology, Singapore: Mouse preimplantation. Preimplantation development.
Sun, X., University of Wisconsin, Madison: Mouse lung development and disease.
Takahashi, J., University of Texas Southwestern Medical Center, Dallas: Mouse circadian clocks and behavior.
Tam, P., Children's Medical Research Institute, Australia: Mouse gastrulation. Mouse neural induction.
Threadgill, D., North Carolina State University, Raleigh: Quantitative trait analysis in mice.
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: Mouse craniofacial development and the neural crest.
Tuveson, D., Cold Spring Harbor Laboratory: Pancreatic cancer.
Wellik, D., University of Michigan Medical Center, Ann Arbor: Hox genes, development, and cancer.

Ion Channels and Synaptic Transmission

June 5–25

INSTRUCTORS **S. Brenowitz,** NIDCD, National Institutes of Health, Bethesda, Maryland
I. Duguid, University of Edinburgh, United Kingdom
P. Kammermeier, University of Rochester Medical Center, New York

ASSISTANTS **C. Bladen,** University of Calgary, Alberta, Canada
J. Grundemann, Friedrich Miescher Institute, Basel, Switzerland
V. Lu, NIH/NIAAA/LMP, Rockville, Maryland
J. Lueck, University of Iowa Carver College of Medicine, Iowa City
M. Rigby, University College London, United Kingdom
J. Schiemann, University of Edinburgh, United Kingdom
N. Wanaverbecq, Aix-Marseille University, Marseille, France

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) were activated by neurotransmitter at central and peripheral synapses, (2) were activated by voltage changes in axons and dendrites, (3) responded to neuromodulators with changes in functional properties, (4) are developmentally required and regulated, or (5) are light-gated and engineered to express in specific neural subtypes. The research interests of guest lecturers reflected these areas of emphasis.

The laboratory component of the course introduced students to state-of-the-art electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. Different recording configurations were used (e.g., whole-cell,

cell-attached, dendritic patches, and voltage- and current-clamp configurations) 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

Chiu, I., Ph.D., Boston Children's Hospital, Massachusetts
Cummings, K., B.A., University at Buffalo, New York
Engl, E., B.A., M.S., University College London, United Kingdom
Furth, K., B.S., National Institutes of Health, Bethesda, Maryland
Harde, E., Diploma, Buchmann Institute for Molecular Life Sciences, Germany
Kim, C., Ph.D., Howard Hughes Medical Institute at Janelia Farm, Ashburn, Virginia
Lazzerini Ospri, L., M.S., Johns Hopkins School of Medicine, Baltimore, Maryland
Murali, S., B.Sc., McMaster University, Hamilton, Canada
Pablo, J.L., B.S., Duke University, Durham, North Carolina
Ruckh, T., Ph.D., Northeastern University, Boston, Massachusetts
Steinberg, E., B.A., University of California, San Francisco
Wei, Q, (Eric), B.S.E, Duke University, Durham, North Carolina

SEMINARS

Boyden, E., Massachusetts Institute of Technology, Cambridge: Optogenetics, in vivo robotics, and other neural circuits tools.
Carandini, M., University College London, United Kingdom: Probing the roles of inhibition in vivo.
Clapham, D., Children's Hospital Boston/Harvard Medical School, Massachusetts: Measuring ion channels in tough places.
Colquhoun, D., University College London, United Kingdom: Is "allosteric" a useful word? What single ion channel measurements say.
Hausser, M., University College London, United Kingdom: Dendritic integration.
Hull, C., Duke University, Durham, North Carolina: Functional properties of fast synaptic inhibitory circuits in the CNS.
Ikeda, S., National Institute on Alcohol Abuse & Alcoholism, Rockville, Maryland: Nutrient substrates, channel modulation, and human genomics: GPR41/FFAR3 in the sympathetic nervous system.
Isom, L.L., University of Michigan, Ann Arbor: Role of sodium channel SCN1A and SCN1B in inherited epilepsy.
Larsson, P., University of Miami, Florida: Molecular mechanism of voltage-gated proton channels.
Lee, A., University of Iowa, Iowa City: Decalmodulation of voltage-gated Ca^{2+} channels.
Nolan, M., University of Edinburgh, United Kingdom: Synaptic mechanisms for computation of location.
Plested, A., Leibnitz Institute for Molecular Pharmacology, Berlin, Germany: Glutamate receptor activation mechanisms.
Shepherd, G., Northwestern University, Chicago, Illinois: Circuit analysis in the neocortex.
Sjostrom, J., McGill University, Montreal Quebec, Canada: Synaptic plasticity and LTP. Spike-timing-dependent plasticity in neocortex.
Xu-Friedman, M., University of Buffalo, SUNY, New York: Synaptic transmission and short-term plasticity.

Vision: A Platform for Linking Circuits, Perception, and Behavior

June 12–25

INSTRUCTORS **F. Briggs,** Geisel School of Medicine, Dartmouth, New Hampshire
A. Huberman, University of California, San Diego

ASSISTANT **O. Dhande,** University of California, San Diego

The purpose of this course was to bring together students and faculty for in-depth and high-level discussions of modern approaches for probing how specific cell types and circuits give rise to defined categories of perception and action. It was also designed to address novel strategies aimed at overcoming diseases that compromise sensory function.

The visual system is the most widely studied sensory modality. Recently, three major shifts have occurred in the field of neuroscience. First, because of the large array of genetic techniques available in mice and the relative ease of imaging and recording from the cortex of small rodents, the mouse visual system has become a premiere venue for attacking the fundamental unresolved question of how specific cells and circuits relate to visual performance at the receptive field and whole-animal level. Second, genetic and viral methods have evolved to the point where neurophysiologists can directly probe the role of defined circuits in species such as macaque monkeys, thus bridging the mechanism-cognition gap. Third, the field of visual neuroscience is rapidly paving the way for widespread clinical application of stem cell, gene therapy, and prosthetic devices to restore sensory function in humans.

The time is ripe to build on the classic paradigms and discoveries of visual system structure, function, and disease to achieve a deep, mechanistic understanding of how receptive fields are organized and filter sensory information, how that information is handled at progressively higher

levels of neural processing, and how different circuits can induce defined categories of percepts and behaviors in the healthy and diseased brain.

This course was supported by the Howard Hughes Medical Institute.

PARTICIPANTS

Baragli, C., B.S., University College London, United Kingdom
Bos, R., Ph.D., University of California, Berkeley
Burbridge, T., M.Phil., Yale University, New Haven, Connecticut
Daum, J., M.S., Friedrich Miescher Institute, Basel, Switzerland
Denman, D., Ph.D., University of Pennsylvania, Philadelphia
Fransen, J., Ph.D., University of Louisville School of Medicine, Kentucky
Garner, A., Ph.D., Allen Institute for Brain Science, Seattle, Washington
Hasse, J., Ph.D., Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire
Hoy, J., Ph.D., University of Oregon, Eugene
Jeurissen, D., Ph.D., Netherlands Institute for Neuroscience, Amsterdam
Kandler, S., Ph.D., IMEC-NERF, Leuven, Belgium
Liberman, A., Ph.D., University of California, Berkeley
Marques, T., Ph.D., Champalimaud Foundation, Lisbon, Portugal
Mock, V., B.A., Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire
Pinto-Teixeira Sousa, F., Ph.D., New York University, New York
Ribic, A., Ph.D., Yale University, New Haven, Connecticut
Rudiger, P., Ph.D., University of Edinburgh, United Kingdom
Stevens, J.-L., M.S./Ph.D., University of Edinburgh, United Kingdom
Tschetter, W., Ph.D., University of Oregon, Eugene
Vaiceliunaite, A., Ph.D., University of Tuebingen, Germany
Wilk, M., Ph.D., Medical College of Wisconsin, Milwaukee
Zhou, Y., M.S., Columbia University, New York

SEMINARS

Berson, D., Brown University, Providence, Rhode Island: Retinal output channels.
Briggman, K., National Institutes of Health, Bethesda, Maryland: Retinal circuitry.
Briggs, F., Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire: Introduction: V1 and beyond.
Callaway, E., The Salk Institute for Biological Studies, San Diego, California: Circuits and functions of cell types and visual areas in mice and monkeys.
Cang, J., Northwestern University, Evanston, Illinois: Critical period plasticity and binocular matching in the mouse visual cortex.
Carandini, M., University College London, United Kingdom: Probing circuits and computations in primary visual cortex.
Chichilnisky, E.J., The Salk Institute, San Diego, California: Visual signaling in primate retina and design of retinal prostheses.
Churchland, A., Cold Spring Harbor Laboratory: Integrating visual information across time and with other modalities.
Clandinin, T., Stanford University, California: Dissecting the circuit mechanisms of visual computation in fruit flies.
Fitzpatrick, D., Max-Planck Florida Institute, Jupiter: Building cortical representations with experience: Insights from visual cortex.
Gandhi, S., National Biological Standards Laboratory, United Kingdom: The mechanisms of critical period plasticity.
Guido, W., University of Louisville, Kentucky: Mouse visual thalamus: Structure, function, and circuitry.
Horwitz, G., University of Washington, Seattle: Cortex II (coding and processing).
Huberman, A., University of California, San Diego: Introduction: Retina to V1.
Maunsell, J., Harvard Medical School, Boston, Massachusetts: Cortical mechanisms of attention.
Movshon, J.A., New York University, New York: Elements of form and motion processing in early visual cortex.
Neitz, M., University of Washington, Seattle: Cone opsin genetics and the cure and prevention of eye disease.
Niell, C., University of Oregon, Eugene: Cell types and receptive fields in the mouse visual system.
Nielsen, K., Johns Hopkins University, Baltimore, Maryland: Cortex I (mapping circuits/alternate pathways).
Pearson, R., University College London, United Kingdom: Retinal repair.
Roska, B., Friedrich Miescher Institute, Basel, Switzerland: Cell-type-specific computations in retina and cortex: Understanding and repair.
Tsao, D., California Institute of Technology, Pasadena: The ventral stream.
Ursey, W.M., University of California, Davis: Functional properties of neural circuits for vision.

Statistical Methods for Functional Genomics

June 21–July 3

INSTRUCTORS **N. Altman,** Penn State University, University Park, Pennsylvania
H. Bussemaker, Columbia University, New York
S. Davis, National Institutes of Health, Bethesda, Maryland
O. Elemento, Weill Cornell Medical College, New York

ASSISTANTS **V. FitzPatrick,** Columbia University, New York
P. Kuruppumullage Don, Penn State University, State College, Pennsylvania
Y. Lee, Penn State University, University Park, Pennsylvania
T. Riley, Columbia University, New York

During the past decade, high-throughput assays have become pervasive in biological research due to both rapid technological advances and decreases in overall cost. To properly analyze the large data sets generated by such assays and thus make meaningful biological inferences, both experimental and computational biologists must understand the fundamental statistical principles underlying analysis methods. This course was designed to build competence in statistical methods for analyzing high-throughput data in genomics and molecular biology.

Detailed lectures and presentations by instructors and guest speakers were combined with hands-on computer tutorials. The methods covered in the lectures were applied to example high-throughput data sets.

This course was supported with funds provided by National Institute of General Medical Sciences.

PARTICIPANTS

Chaudhuri, R., Ph.D., Garvan Institute for Medical Research, Darlinghurst, Australia
Demel, C., M.S., Ludwig-Maximilians-Universtat, Munich, Germany
Dose, M., Ph.D., National Institutes of Health, Bethesda, Maryland
Durresi, E., M.S., Harvard University, Boston, Massachusetts
Francescatto, M., M.S., German Center for Neurodegenerative Diseases, Tuebingen, Germany
Franco, L., M.D., Baylor College of Medicine, Houston, Texas
Fufa, T., Ph.D., National Institutes of Health, Bethesda, Maryland
Gearhart, M., Ph.D., University of Minnesota, Minneapolis
He, X., Ph.D., National Cancer Institute, NIH, Bethesda, Maryland
Jaksik, R., M.S., Silesian University of Technology, Gliwice, Poland
Jeselsohn, R., M.D., Dana-Farber Cancer Institute, Boston, Massachusetts
Liu, X.-Y., Ph.D., University of Wisconsin, Madison
Lopez-Anido, C., B.A., University of Wisconsin, Madison
Mehta, N., B.S., Northwestern University, Chicago, Illinois
Mitchell, A., Ph.D., Mount Sinai School of Medicine, New York
Sawicka, A., M.S., Medical University of Vienna, MFPL, Austria
Shetty, A., M.S., University of Baltimore, Maryland
Sun, W., M.S., University of Rochester Medical Center, New York
Sweet-Cordero, E.A., M.D., Stanford University, California
Williams, K., B.A., Washington University, St. Louis, Missouri
Wirthlin, M., B.A., Oregon Health & Science University, Portland
Zhao, G., Ph.D., SUNY, Stony Brook, New York
Zimmers, T., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Zou, Y., B.S., Memorial Sloan-Kettering Cancer Center, New York

SEMINARS

Carlson, M., Fred Hutchinson Cancer Research Center, Seattle, Washington: Bioconductor annotation and data resources.
Davis, S., National Institutes of Health, Bethesda, Maryland: R precourse.
Gerstein, M., Yale University, New Haven, Connecticut: Grappling with a big data blizzard in genomics: Reproducible and open annotation, networks, models, and tools.
Hodges, E., Cold Spring Harbor Laboratory: Domains of DNA hypomethylation are pockets of activity for genome regulation and organization.
Horvath, S., University of California, Los Angeles: Empirical evaluation of prediction and correlation network methods applied to genomic data.
Ingolia, N., Carnegie Institution, Baltimore, Maryland: Genome-wide profiling of translation initiation and protein.
Leslie, C., Memorial Sloan-Kettering Cancer Center, New York: New insights into "regulatory transcriptomics" with CLIP-Seq and 3′-Seq.
Reimers, M., VCU School of Medicine, Richmond, Virginia: Genomics of the human brain.

Workshop on Pancreatic Cancer

June 26–July 2

INSTRUCTORS **D. Bar-Sagi,** New York University Medical Center, New York
S. Leach, Johns Hopkins University School of Medicine, Baltimore, Maryland
D. Tuveson, Cold Spring Harbor Laboratory

ASSISTANT **J. Bailey,** Johns Hopkins University, Baltimore, Maryland

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-on-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.

This course was supported equally by the Lustgarten Foundation and the Pancreatic Cancer Action Network.

PARTICIPANTS

Badgley, M., B.A., Columbia University, New York
Bartholin, L., Ph.D., Cancer Research Center of Lyon (CRCL), Lyon, France
Bien, H., Ph.D., Stony Brook University, Stony Brook, New York
Egeblad, M., Ph.D., Cold Spring Harbor Laboratory
Gomes-DaGama, E.G., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Gore, J., Ph.D., Indiana University, Indianapolis

Handler, J., Ph.D., New York University School of Medicine, New York
Hendley, A., Ph.D., Johns Hopkins University, Baltimore, Maryland
Houghton, J., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Kim, M., M.D., University of Texas M.D. Anderson Cancer Center, Houston
Liu, X., Ph.D., Eppley Institute, University of Nebraska Medical Center, Omaha
Park, J., Ph.D., Seoul National University Hospital, South Korea
Quinn, B., M.S., Virginia Commonwealth University, Richmond
Sherman, M., Ph.D., Salk Institute, La Jolla, California
Schmid, M., Ph.D., Lecturer and Research Group Leader, Liverpool, United Kingdom
Shields, M., Ph.D., Cold Spring Harbor Laboratory
Smith, K., Ph.D., Mayo Clinic, Jacksonville, Florida
Sugumar, A., M.Ph., University of Kansas, Kansas City
Takahashi, R., B.S., The University of Tokyo, Japan
Takeuchi, K., Ph.D., Mayo Clinic, Jacksonville, Florida
Waghray, M., Ph.D., University of Michigan, Ann Arbor
Yoon, S., Ph.D., Beckman Research Institute of City of Hope, Duarte, California

SEMINARS

Bar-Sagi, D., New York University Medical Center, New York; Leach, S., Johns Hopkins University School of Medicine, Baltimore, Maryland; Tuveson, D., Cold Spring Harbor Laboratory: In vivo and in vitro models.
Baylin, S., Johns Hopkins University School of Medicine, Baltimore, Maryland: Genetics I.
Crawford, H., Mayo Clinical Cancer Center, Rochester, Minnesota: Animal models of pancreatitis and the epithelial contributions and responses.
Der, C., University of North Carolina, Chapel Hill: Targeting K-Ras for pancreatic cancer treatment.
Hollingsworth, T., University of Nebraska, Omaha: Clinical trials.
Iacobuzio-Donahue, C., Johns Hopkins University School of Medicine, Baltimore, Maryland: Current concepts in pancreatic cancer genetics.
Kelly, K., University of Virginia, Charlottesville: Molecular imaging and biomarker discovery in PDAC.
Kimmelman, A., Dana-Farber Cancer Institute, Boston, Massachusetts: Pancreatic cancer metabolism.
Klimstra, D., Memorial Sloan-Kettering Cancer Center, New York: Pathology of pancreatic ductal carcinoma and other solid neoplasms.
Klimstra, D., Memorial Sloan-Kettering Cancer Center, New York: Pathology of cystic and intraductal neoplasms.
Leach, S., Johns Hopkins University School of Medicine, Baltimore, Maryland: Developmental biology and PDA.
Megibow, A., New York University, New York: Imaging pancreatic cysts.
Megibow, A., New York University, New York: The altered metabolism of pancreatic cancer.
Miller, G., New York University Langone Medical Center, New York: Pancreatic inflammation: A historical and clinical perspective. Cellular and biochemical mediators of stromal inflammation from pancreatitis to carcinogenesis.
Murtaugh, C., Harvard Medical School, Cambridge, Massachusetts: A cellular "identity crisis" in pancreatic cancer initiation.
Olive, K., Columbia University, New York: Preclinical therapeutics in genetically engineered models of pancreatic cancer.
Simeone, D., University of Michigan Medical Center, Ann Arbor: Clinical evaluation. Surgical management of pancreatic cancer.
Ting, D., Massachusetts General Hospital/Harvard Medical School, Boston: Early detection biomarkers for pancreatic cancer.
Vander Heiden, M., Massachusetts Institute of Technology, Cambridge: Metabolomics: Role of metabolism in pancreatic cancer.
Vonderheide, R., University of Pennsylvania, Philadelphia: Immunobiology of pancreatic cancer.
Watson, J., Cancer Biology, National Cancer Institute, Bethesda, Maryland: Funding opportunities; training opportunities.
Yu, K., Memorial Sloan-Kettering Cancer Center, New York: Clinical trials in pancreatic cancer.

Drosophila Neurobiology: Genes, Circuits, and Behavior

June 28–July 16

INSTRUCTORS **G. Macleod,** University of Texas Health Science Center, San Antonio
K. O'Connor-Giles, University of Wisconsin, Madison
A. Rothenfluh, University of Texas Southwestern, Dallas

ASSISTANTS **J. Bruckner,** University of Wisconsin, Madison
S. Gratz, University of Wisconsin, Madison
J. Jin, University of Texas Southwestern, Dallas
A. Rossano, University of Texas Health Science Center, San Antonio

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 two and a half-week course was designed to introduce students to a wide variety of topics and techniques, including the latest approaches in studying nervous system development, activity, and connectivity, as well as complex behaviors and disease models. Daily research seminars presented comprehensive overviews of specific subfields of nervous system function or focused on specific techniques and approaches to study fly neurobiology. Expert guest lecturers discussed their findings and approaches, and brought along their own assays and techniques for students to learn in the laboratory part of the course. The hands-on portion of the course was centered around student-led projects, where gene mutants were discovered and analyzed throughout the course using the different morphological and physiological measurements and behavioral paradigms at hand. This included electrophysiological and in vivo calcium

recordings and anatomical examination and circuit mapping, as well as numerous quantitative behavioral measures. 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 on Drug Abuse, the National Institute of Neurological Disorders and Stroke, and the National Science Foundation.

PARTICIPANTS

Allo, M., Ph.D., European Molecular Biology, Heidelberg, Germany
Clark, M., B.S., University of Oregon, Eugene
Imler, E., Ph.D., University of Arizona, Tucson
Kreko, T., B.S., University of Texas Health Science Center, San Antonio
Lembke, K., B.A., Oregon Health and Science University School of Dentistry, Portland
Macara, A.M., B.S., University of Michigan, Ann Arbor
Muenzel, J., Ph.D., University of Edinburgh, United Kingdom
Namiki, S., Ph.D., Howard Hughes Medical Institute, Ashburn Virginia
Pankova, K., B.Sc., Max-Planck Institute of Neurobiology, Martinsried, Germany
Shields, M., B.S., Colorado State University, Fort Collins
Titlow, J., M.S., University of Kentucky, Lexington
Yang, L., B.Sc., University of Oxford, United Kingdom

SEMINARS

Daniels, R., University of Wisconsin, Madison: NMJ physiology.
Dubnau, J., Cold Spring Harbor Laboratory: Learning and memory.
Kaun, K., HHMI/Janelia Farm Research Campus, Ashburn, Virginia: Flies and ethanol.
Keene, A., University of Reno, Nevada: Circadian rhythms/sleep.
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 dense core vesicles and neurosecretion.
MacLeod, G., University of Texas Health Science Center, San Antonio: Ca^{++} and pHluorin imaging.
McKellar, C., HHMI/Janelia Farm Research Campus, Ashburn, Virginia: Genetic tools for mapping circuits underlying behavior.
Nitabach, M., Yale School of Medicine, New Haven, Connecticut: Genetically encoded voltage indicators.
O'Connor-Giles, K., University of Wisconsin, Madison: Synaptogenesis.
Pulver, S., Janelia Farms, Ashburn Virginia: CNS physiology.
Rolls, M., Penn State, University Park, Pennsylvania: Neuronal polarity.
Rothenfluh, A., University of Texas Southwestern, Dallas: Studying behavior in *Drosophila*.
Simpson, J., HHMI/Janelia Farm Research Campus, Ashburn, Virginia: Genetic tools for mapping circuits underlying behavior.
Simpson, J., HHMI/Janelia Farm Research Campus, Ashburn, Virginia: Genetic tools for mapping circuits underlying behavior.
Strausfeld, N., University of Arizona, Tucson: Comparative neuroanatomy.
Turner, G., Cold Spring Harbor Laboratory: Studying neural activity in the CNS.
White, B., National Institute of Mental Health, Bethesda, Maryland: Circuit mapping complex behaviors.
Yoshihara, M., University of Massachusetts Medical School, Worcester: *Drosophila* feeding circuit.
Zwart, M., University of Cambridge, United Kingdom: CNS physiology.

Frontiers of Techniques in Plant Science

June 28–July 18

INSTRUCTORS
S. Harmer, University of California, Davis
M. Johnson, Brown University, Providence, Rhode Island
R. Last, Michigan State University, E. Lansing
J. Maloof, University of California, Davis
S.-H. Shiu, Michigan State University, East Lansing

ASSISTANTS
A. Burkhardt, Michigan State University, East Lansing
A. Leydon, Brown University, Providence, Rhode Island
G. Moghe, Michigan State University, East Lansing
S. Uygun, Michigan State University, East Lansing
H. Van Houtte, KU Leuven, Heverlee, Belgium

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 development 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

Atkins, P., B.A., University of Minnesota, St. Paul
Englhart, M., M.S., University of Regensburg, Germany
Gentzel, I., Ph.D., Ohio State University, Columbus
Gonzalez, T., B.S., University of California, Berkeley
Jahnsen, M., M.A., University of Copenhagen, Denmark
Jawahir, V., Ph.D., University of Missouri, St. Louis
Munoz Sanhueza, L.G., Lic., Norwegian University of Life Sciences, Ås, Norway
Noble, J., B.S., University of Arizona, Tucson
Pater, D., Ph.D., University of San Diego, La Jolla, California
Schroeder, S., Ph.D., University of Oklahoma, Norman
Seraj, Z., Ph.D., University of Dhaka, Bangladesh
Siler, E., B.S., Michigan State University, E. Lansing
Stonoha, C., B.S., University of Massachusetts, Amherst
Tulin, F., Ph.D., The Rockefeller University, New York
Zimmerman, N., Ph.D., Stanford University, California

SEMINARS

Brady, S., University of California, Davis: Root development.
Dinesh-Kumar, S., University of California, Davis: Plant immune response.
Gehring, M., Whitehead Institute, Cambridge, Massachusetts: Epigenetics.
Geitmann, A., University of Montreal, Canada: Overview of the use of modeling in plant science. Size, speed, and power: How the male gametophyte delivers the sperm cells in the flowering plants.
Grossniklaus, U., University of Zurich, Switzerland: Reproductive development.
Harmer, S., University of California, Davis: Circadian rhythm.
Johnson, M., Brown University, Providence, Rhode Island: Overview of genetic approaches in plant. Analysis of male reproductive development and function. Imaging and plant biology.
Kellogg, E., University of Missouri, St. Louis: Molecular systematics and developmental genetics.
Last, R., Michigan State University, E. Lansing: Specialized metabolites. Large-scale genetics/phenomics.
Maloof, J., University of California, Davis: Light signaling. Quantitative trait loci.
McSteen, P., University of Missouri, Columbia: Meristem development.
Noel, J., HHMI/Salk Institute for Biological Sciences, La Jolla, California: Plant metabolism.
Oldroyd, G., John Innes Center, Norwich, United Kingdom: Plant symbiotic associations.
Osborn, T., Monsanto, Chesterfield, Missouri: Genetic variation we should care about, and why?
Pappin, D., Cold Spring Harbor Laboratory: Mass spectrometry.
Provart, N., University of Toronto, Ontario, Canada: Bioinformatic tools in plant research.
Raikhel, N., University of California, Riverside: The secretory system.
Shiu, S.-H., Michigan State University, E. Lansing: Evolutionary genomics.
Sinha, N., University of California, Davis: Introduction to plant anatomical development.
Spalding, E., University of Wisconsin, Madison: Kinematics.
Tiffin, P., University of Minnesota, Minneapolis: Population genetics/genomics.
Timmermans, M., Cold Spring Harbor Laboratory: RNA interference.
Voytas, D., University of Minnesota, Minneapolis: Precise genome engineering with sequence-specific nucleases.
Walling, L., University of California, Riverside: Plant-insect interaction.

Advanced Techniques in Molecular Neuroscience

July 2–18

INSTRUCTORS

C. Lai, Indiana University, Bloomington
R. Lansford, California Institute of Technology, Pasadena
J. Loturco, University of Connecticut, Storrs

ASSOCIATE INSTRUCTORS

J. Dougherty, Washington University, St. Louis, Missouri
K. Haas, University of British Columbia, Canada
B. Maher, Johns Hopkins Medical School, Baltimore, Maryland
J. Singh, University of Pennsylvania, Philadelphia

TEACHING ASSISTANTS

F. Chen, University of Connecticut, Storrs
J. Dalal, Washington University, St. Louis, Missouri
M. Girgenti, University of Connecticut, Storrs
D. Huss, California Institute of Technology, Pasadena
H. Kang, Harvard University, Cambridge, Massachusetts
A. Kaur, Indiana University, Bloomington
S. Maloney, Washington University School of Medicine, St. Louis, Missouri
J. Morris, University of Pennsylvania, Philadelphia
K. Podorski, University of British Columbia, Canada
M. Rannals, Lieber Institute for Brain Development, Baltimore, Maryland
A. Zybura, 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 (siRNA) for regulating the expression of specific genes in neurons; practical exercises in gene delivery systems including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use and design of BAC transgenic vectors; quantitative real time PCR analyses from small numbers of cells (RNA purification, PCR optimization, interpretation of results); and application of RNA amplification (aRNA) for single-cell analysis. Each laboratory module was followed by presentations and discussions focused on data interpretation, protocol troubleshooting, and ways to modify and potentially improve existing techniques.

This course was supported with major funding provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Akahori Stroud, H., B.A., Harvard Medical School, Boston, Massachusetts
Bundo, M., Ph.D., The University of Tokyo, Japan
Coles, C., Ph.D., German Centre for Neurodegenerative Disease-DZNE, Bonn, Germany
Cruz, E., B.S., Ponce School of Medicine & Health Sciences, Ponce, Puerto Rico
Franquinho, F., B.Sc., Instituto de Biologia Molecular e Cellular, Porto, Portugal
Fuchs, C., M.S., University of Bologna, Italy
Gabreski, N., B.S., University of Michigan, Ann Arbor
Gustafsen, C., Ph.D., Aarhus University, Denmark
Huang, X., B.S., Vanderbilt University, Nashville, Tennessee
Inoue, W., Ph.D., University of Calgary, Canada
Kopec, A., B.S., New York University, New York
Lang, J., Ph.D., University of California, Davis/Shriners' Hospital, Sacramento
Sabo, J., Ph.D., University of California, San Francisco
Schank, J., B.A., National Institute for Alcohol Abuse & Alcoholism, Bethesda, Maryland
Sloan, S., B.S., Stanford University, California
Yu, J., B.A., Max-Planck Florida Institute, Jupiter

SEMINARS

Darnell, R., The Rockefeller University, New York: Next-generation sequencing and RNA genomics.
Eberwine, J., University of Pennsylvania, Philadelphia: Single-cell neurogenomics: Insights into neuronal functioning.
Haas, K., University of British Columbia, Vancouver, Canada: Distinguished ATMN Alumnus Lecture: In vivo imaging of developmental brain plasticity and metaplasticity.
Huang, J., Cold Spring Harbor Laboratory: Genetic dissection of neural circuits in neocortex: Chandeliers light up the path.
Josselyn, S., University of Toronto, Canada: Making, breaking, and linking fear memories in mice.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Talking about Science. Connectomics.
Zhang, F., Massachusetts Institute of Technology, Cambridge: Neuroengineering: Molecular and optical axes of control.

Proteomics

July 9–24

INSTRUCTORS
M. Bereman, University of Washington, Seattle
M. Cilia, USDA-ARS, Ithaca, New York
I. Cristea, Princeton University, New Jersey
D. Pappin, Cold Spring Harbor Laboratory

ASSISTANTS
S. DeBlasio, USDA-ARS, Ithaca, New York
H. Budayeva, Princeton University, New Jersey
B. Diner, Princeton University, New Jersey
T. Greco, Princeton University, New Jersey
D. Igwe, USDA-ARS, Ithaca, New York
A. Kliot, The Volcani Institute, Jerusalem, Israel
M. Sweeney, Boyce Thompson Institute, Ithaca, New York

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 was supported with funds provided by the National Institute of Child Health and Human Development.

PARTICIPANTS

Balasubramani, A., Ph.D., La Jolla Institute for Allergy and Immunology, California
Bantysh, O., M.S., Institute of Gene Biology RAS, Moscow, Russia
Chai, H., B.A., University of California. Los Angeles
Colpan, M., B.S., Washington State University, Pullman
Cook, C., Ph.D., Colorado State University, Fort Collins
Daniels, C., Ph.D., Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland
Duncombe, T., B.S., University of California, Berkeley
Gallo MacFarlane, E., Ph.D., Johns Hopkins University, Baltimore, Maryland
Huang, H., Ph.D., Wilmer Eye Institute, Baltimore, Maryland
Johnson, E., Ph.D., Washington University, St. Louis, Missouri
Kong, L., M.S., McGill University, Montreal, Canada
Patel, A., B.Sc., McGill University, Ste. Anne de Bellevue, Canada
Polanco, G., B.S., University of Texas. El Paso
Tong, Z., B.S., Cornell University, Ithaca, New York
Womble,T., Ph.D., Florida A&M University, Tallahassee

SEMINARS

Bereman, M., University of Washington, Seattle: Introduction to MS.
Bruce, J., University of Washington, Seattle: Protein interaction topologies.
Cilia, M., USDA-ARS, Ithaca, New York: Applications of 2-D DIGE technology.
Clauser, K., Broad Institute/Massachusetts Institute of Technology, Cambridge: Manual de novo peptide MS/MS interpretation for evaluating database search results.
Colon, F., University of North Carolina, Chapel Hill: Isolation of endogenous protein complexes from early mouse embryos.
Cristea, I., Princeton University, New Jersey: Protein interactions.
MacCoss, M., University of Washington, Seattle: Introduction to peptide quantification. Introduction to targeted method refinement with Skyline. Removing the subjectivity of peptide list.
Medzihradsky, K., University of California, San Francisco: How to interpret/evaluate MS/MS spectra: Focus on PTM assignments–I. How to interpret/evaluate MS/MS spectra: Focus on PTM assignments–II.
Muddiman, D., North Carolina State University, Raleigh: ESI MALDI and hybrids of the two. Labeling with isotopic glycan hydrazide tugs (INLIGHT): A novel glycan relative quantification strategy, development, and application. Mass spectrometry-based absolute protein quantification.
Pappin, D., Cold Spring Harbor Laboratory: Tandem MS identification of proteins.
Smolka, M., Cornell University, Ithaca, New York: Signaling networks.
Wolf-Yadlin, A., University of Washington, Seattle: Phosphoproteomics and cell signaling.

Biology and Disorders of Learning and Memory

July 20–August 2

INSTRUCTORS **A. Duarte,** Georgia Institute of Technology, Atlanta
F. Froemke, New York University School of Medicine, New York
K. Martin, University of California, Los Angeles
J. Raymond, Stanford University, California

ASSISTANTS **N. Amin,** University of California, Berkeley
I. Carcea, New York University, New York

This lecture course provided an introduction to cellular, molecular, and systems approaches to learning and memory. It was suited for graduate students and postdoctoral fellows in molecular biology, neurobiology, and psychology, as well as research workers who are interested in an introduction to this field. The course covered topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of modern behavioral studies of learning and memory, the cell and molecular biology of neuronal plasticity, cellular and molecular mechanisms of simple forms of learning and memory, and systems approaches to learning in vertebrates and humans. Lectures were complemented by exercises in which students worked in small groups with lecturers to discuss topical issues in learning and memory, to evaluate recent studies, and to identify and formulate new research questions and approaches. The course was thus designed not only to introduce students to the field of learning and memory, but also to provide an intellectual framework upon which future studies can be built.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

An, L., Ph.D., Xidian University, Xi'an, Shaanxi Province, China
McCue, M., B.A., Research Foundation for Mental Hygiene/ Nathan Kline Institute, Orangeburg, New York
Baj, G., Ph.D., Ph.D., University of Trieste, Italy
Pastuzyn, E., Ph.D., University of Utah, Salt Lake City
DiMauro, A., M.D./Ph.D., Boston University, Massachusetts
Robinson, N., Ph.D., Boston University, Massachusetts
Dulas, M., M.S., Georgia Institute of Technology, Atlanta
Sukhanova, A., Ph.D., Kurchatov NBIC Center, Moscow, Russia
Guo, W., Ph.D., Massachusetts Eye and Ear Infirmary, Boston
Tamtè, M., M.Sc., Lund University, Sweden
Ibrahim, M.S., King Abdullah for Science and Technology, Thuwal, Saudi Arabia
Templer, V., Ph.D., Emory University, Atlanta, Georgia
Kau Escalante, M., M.S., Pierre-and-Marie-Curie University, Paris, France
Turney, I., Ph.D., Pennsylvania State University, University Park
Kim, J., B.A., Nathan Kline Institute, Orangeburg, New York
Waller, H., M.S., Montana State University, Bozeman
Lebois, E., Ph.D., Emory University, Atlanta, Georgia
Zalcman, G., M.S., IFIByNE-CONICET, Buenos Aires, Argentina
Wei-Jye, L., Ph.D., Icahn School of Medicine at Mount Sinai, New York

SEMINARS

Barth, A., Carnegie Mellon University, Pittsburgh, Pennsylvania: Cumulative experience-dependent plasticity in neocortical circuits.
Duarte, A., Georgia Institute of Technology, Atlanta: What can neuroimaging contribute to our understanding of age-related memory changes?
Eichenbaum, H., Boston University, Massachusetts: Memory mechanisms and the hippocampus.
Hensch, T., Harvard University, Boston, Massachusetts: Biological constraints on brain plasticity.
Josselyn, S., Hospital for Sick Children/University of Toronto, Canada: Molecular, cellular, and systems basis of fear learning,
Levey, A., Emory University School of Medicine, Atlanta, Georgia: Alzheimer's disease research and discovery.
Martin, K., University of California, Los Angeles: Regulating gene expression in neurons during synapse formation and synaptic plasticity.
Moscovitch, M., University of Toronto, Canada: Recent and remote memory in humans and rodents.
Raymond, J., Stanford University, Massachusetts; Froemke, R., New York University School of Medicine: Synaptic plasticity in vitro and in vivo.
Squire, L., University of California/Veterans Affairs Medicine Center, San Diego; Neuroscience of memory in historical perspective: 1881–2005. Conscious and unconscious memory systems of the mammalian brain.
Turner, G., California Institute of Technology, Pasadena: Pavlovian learning in *Drosophila*.

Computational Cell Biology

July 23–August 12

INSTRUCTORS
R. Albert, Pennsylvania State University, University Park
L. Loew, University of Connecticut Health Center, Farmington
G. Smith, The College of William and Mary, Williamsburg, Virginia

ASSISTANTS
S.-A. Brown, Mayo Clinic, Rochester, New York
K. Hardcastle, William and Mary College, Williamsburg, Virginia
L. Harris, University of Pittsburgh School of Medicine, Pennsylvania
S. Jalil, University of Texas Health Center, Houston
O. Marchenko, University of Connecticut Health Center, Farmington
X. Wang, The College of William and Mary, Williamsburg, Virginia
S. Weinberg, The College of William and Mary, Williamsburg, Virginia

Computational cell biology was the field of study that applied the mathematics of dynamical systems together with computer simulation techniques to the study of cellular processes. The field encompassed 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, e.g., 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 underlies 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

Chen, Y., B.S., West Virginia University, Morgantown
Connolly, N., B.E., Royal College of Surgeons, Dublin, Ireland
Daneshjou, N., B.S., University of Illinois, Chicago
Ding, Q., A.B.S, University of Pittsburgh, Pennsylvania
Dolatshahi, S., B.S., Georgia Institute of Technology, Atlanta
Ghochani, M., B.S., NICHD, National Institutes of Health, Bethesda, Maryland
Grande, A., B.A., CONICET-UBA, Ciudad Autonoma de Buenos Aires, Argentina
Imran Alsous, J., M.S., Princeton University, New Jersey
Lee, W., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Matthews, M., B.S., North Carolina State University, Raleigh
Morin, M., B.A., LISBP, Toulouse, France
Norman, R., B.S., University of Connecticut Health Center, Farmington
Ravi, B., B.Tech., CSIR-Institute of Genomics & Integrative Biology, New Delhi, India
Suarez, I., M.A., University of Miami Miller School of Medicine, Florida
van Mourik, T., M.Sc., Utrecht University, The Netherlands
Yang, Y., Ph.D., Emporia State University, Emporia, Kansas

SEMINARS

Albert, R., Pennsylvania State University, University Park: Graph theoretical analysis of intracellular networks. Linking the structure and dynamics of intracellular networks. Discrete dynamic modeling of signal transduction networks.
Chen, T., Stanford University, California: Cell cycle modeling.
Cohens, D.; Samant, A., Mathworks, Inc., Natick, Massachusetts: Matlab Overview. Matlab sim biology.
DeVries, G., University of Alberta, Edmonton, Canada: Enzyme kinetics I: Michaelis-Menten kinetics. Enzyme kinetics II: Cooperativity and the Hill equation. Modeling a biochemical network (resulting in a sharp threshold). Modeling a genetic network (resulting in a switch with memory).
Feinberg, M., Ohio State University, Columbus; Chemical reaction network theory.
Harris, L., University of Pittsburgh School of Medicine, Pennsylvania: Rule-based modeling.
Hasty, J., University of California, San Diego, La Jolla: Gene networks.
Hucka, M., California Institute of Technology, Pasadena: Sbml, model databases, and translation.
Keener, J., University of Utah, Salt Lake City; Cardiac models.
Loew, L, University of Connecticut Health Center, Farmington: Virtual cell and ODEs. Actin dendritic nucleation.
Mendes, P., University of Manchester, United Kingdom: Copasi and parameter estimation.
Moraru, I., University of Connecticut Health Center, Farmington: Model building with virtual cells. Virtual cell stochastic reactions.
Phair, R., Integrative Bioinformatics, Sunnyvale, California: Fluorescent protein kinetics and model diagrams.
Sachs, K.; Chen, T., Stanford University, California: Bayesian analysis of reaction networks. Machine learning/network analysis.
Schaff, J., University of Connecticut Health Center, Farmington: Virtual cells and PDEs. Virtual cell spatial stochastic.
Smith, G.D., The College of William and Mary, Williamsburg, Virginia: Cellular biophysics and ODEs. Molecular cell biology and ODEs. Random walks/diffusion. Stochastic modeling.
Sobie, E., Icahn School of Medicine at Mount Sinai, New York: Local control.
Terman, D., Ohio State University, Columbus: Dynamical systems.
Tyson, J., Virginia Tech, Blacksburg: Cell cycle.

Eukaryotic Gene Expression

July 23–August 12

INSTRUCTORS
K. Adelman, NIH/NIEHS, Research Triangle Park, North Carolina
W. L. Kraus, University of Texas Southwestern Medical Center, Dallas
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri
D. Taatjes, University of Colorado, Boulder

ASSISTANTS
B. Gibson, University of Texas Southwestern Medical Center, Dallas
D. Gilchrist, NIEHS, Research Triangle Park, North Carolina
G. Fromm, NIH/NIEHS, Research Triangle Park, North Carolina
K. Liang, Stowers Institute for Medical Research, Kansas City, Missouri
Z. Poss, University of Colorado, Boulder

This course was designed for students, postdocs, and principal investigators who have recently ventured into the exciting area of gene regulation. The course focused on state-of-the-art strategies and techniques used in the field. Emphasis was placed both on in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. They isolated transcription factor complexes and assessed their activity in functional 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 learned widely used techniques such as qRT-PCR and chromatin immunoprecipitation (ChIP). They also used RNAi for specific knock-down experiments. Determining cellular gene expression profiles has been accelerated tremendously by microarray and sequencing technology. Students received hands-on training in performing and interpreting results from microarrays, ChIP-Seq, and RNA-Seq data sets.

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. Emphasis was placed on advantages and limitations of specific techniques and data interpretation. The students were encouraged and expected to actively participate in these discussions. Guest lecturers discussed contemporary problems in eukaryotic gene regulation and technical approaches to their solution. From the guest lectures and discussions, students learned to design effective experiments, properly interpret their own data, and critically evaluate the gene expression literature.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Alzrigat, M., M.Sc., Uppsala University, Sweden
Au, E., B.S., Thomas Jefferson University, Philadelphia, Pennsylvania
Audetat, K., B.S., University of Colorado, Boulder
Ben Chaabane, S., M.S., University of Copenhagen, Frederiksberg C, Denmark
Chottekalapanda, R., Ph.D., The Rockefeller University, New York
Mokube Ekumi, K.M., M.S., University of Helsinki, Finland
Fusby, B., B.S., University of Colorado Anschutz Medical Campus, Aurora
Garcia-Molinero, V., M.S., Prince Felipe Research Center, Valencia, Spain
Hanley, M., B.A., Harvard University, Cambridge, Massachusetts
Hill, C., Ph.D., Burke-Cornell Medical Research Institute, White Plains, New York
Malavazi, I., Ph.D., Universidade Federal de Sao Carlos, Sao Paulo, Brazil
Rutledge, E., B.A., Baylor College of Medicine, Houston, Texas
Sapochnik, D., B.S., LFBM-IFIByNE-UBA-CONICET, Buenos Aires, Argentina
Strandjord, D., B.A., University of Minnesota, Minneapolis
Sun, Y., B.S., Mount Sinai School of Medicine, New York
Viadiu, H., Ph.D., University of California, San Diego, La Jolla

SEMINARS

Akhtar, A., Max-Planck Institute of Immunobiology and Epigenetics, Freiburg, Baden-Wuerttemberg, Germany: Dosage compensation: A paradigm to study epigenetic regulation.
Almouzni, G., Institut Curie, Paris, France: Chromatin assembly: Stay tuned!
Espinosa, J.M., HHMI/University of Colorado at Boulder, Denver: Harnessing the p53 transcriptional network for cancer therapeutics.
Furlong, E., European Molecular Biology Laboratory, Heidelberg, Germany: Enhancers: *cis*-Regulatory elements that control the timing and location of gene expression.
Gilmour, D., Penn State University, University Park, Pennsylvania: RNA polymerase II dynamics and promoter proximal pausing.
Johnston, M., University of Colorado, Denver: Feasting, fasting, and fermenting: How yeasts sense glucose.
Kadonaga, J., University of California, San Diego, La Jolla: Peculiarities of promoters, prenucleosomes, and other planetary phenomena.
Kingston, R., Massachusetts General Hospital/Harvard Medical School, Boston: Epigenetic mechanisms: Mapping long noncoding RNAs and modulating chromatin structure.
Levine, M., University of California, Berkeley: The metazoan promoter as a developmental.
Manley, J.; Prives, C., Columbia University, New York: Factors linking gene expression pathways and neurological disease (Manley). The two faces of p53: Tumor suppressor and oncogene (Prives).

Pugh, F., Penn State University, University Park, Pennsylvania: Subnucleosomal chromatin architecture.
Rinn, J., Harvard University, Cambridge, Massachusetts: Through the genome and into noncodarnia.
Roy, A., Tufts University School of Medicine, Boston, Massachusetts: Growth regulatory functions of the transcription factor TFII-I.
Smale, S., University of California School of Medicine, Los Angeles: Transcriptional cascades induced by inflammatory stimuli.
Tyler, J., University of Texas/M.D. Anderson Cancer Center, Houston: Regulation of gene expression by chromatin assembly and disassembly.
Whetstine, J., Massachusetts General Hospital and Harvard University, Charlestown: Looking at cancer through the eyes of histone demethylases.

Yeast Genetics and Genomics

July 23–August 12

INSTRUCTORS **M. Dunham,** University of Washington, Seattle
M. Gartenberg, Robert Wood Johnson Medical School, Piscataway, New Jersey
S. Jaspersen, Stowers Institute for Medical Research, Kansas City, Missouri

ASSISTANTS **S. Burns,** Stowers Institute for Medical Research, Kansas City, Missouri
M. Chen, Robert Wood Johnson Medical School, Piscataway, New Jersey
C. Payen, University of Washington, Seattle

This course was a modern, state-of-the-art laboratory course designed to teach students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical and modern genetic approaches were emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination, as well as array-, next-generation sequencing, and genome-based methods of analysis facilitated by the yeast genome, the deletion collection, and other genomic resources available to the community. Molecular genetic techniques, including yeast transformation, gene replacement by PCR, construction and analysis of gene fusions, and generation of mutations were also emphasized. Students used classical approaches and modern whole-genome sequencing to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality as well as synthetic genetic array (SGA). Students were immersed in yeast genomics and performed and interpreted experiments using DNA arrays and multiplex sequencing. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, GFP-protein fusions, and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental

aspects of yeast genetics were presented along with seminars given by prominent experts in the field on topics of current interest.

This course was supported with funds provided by a generous gift from Dr. David Botstein.

PARTICIPANTS

Aviran, S., Ph.D., University of California, Berkeley
Becker-Kettern, J., M.Sc., University of Luxembourg, Esch-sur-Alzette, Luxembourg
Carvunis, A.-R., Ph.D., University of California, San Diego
Dorrity, M., B.S., University of Washington, Seattle
Garabedian, M., B.S., Brandeis University, Waltham, Massachusetts
Garcia, D., Ph.D., Stanford University School of Medicine, California
Gray, V., B.S., University of Washington, Seattle
Grinberg, G., B.Sc., Tel Aviv University, Israel
Halimi, M., B.S., Brandeis University, Waltham, Massachusetts
Jiang, S., B.S., Tsinghua University, Beijing, China
Ledesma-Fernandez, M.-E., B.Sc., MRC National Institute for Medical Research, London, United Kingdom
Lyon, G., Ph.D., Cold Spring Harbor Laboratory
Morlot, S., Ph.D., University of Geneva, Switzerland
Sanchez de Groot, N., Ph.D., Medical Research Council, Cambridge, United Kingdom
Schwartz, B., Ph.D., Columbus State University, Columbus, Georgia
Strope, P., Ph.D., Duke University, Durham, North Carolina

SEMINARS

Biggins, S., Fred Hutchinson Cancer Research Center, Seattle, Washington: How do cells get the right chromosomes?
Bloom, K., University of North Carolina at Chapel Hill: Dissecting the function of a chromatin spring.
Boeke, J., Johns Hopkins University, Baltimore, Maryland: Synthesizing and scrambling Sc2.0, a designer yeast genome.
Boone, C., University of Toronto, Canada: The genetic landscape of a cell.
Fink, G., Whitehead Institute for Biomedical Research/ MIT, Chestnut Hill, Massachusetts: From genotype to phenotype—An inconvenient truth.
Gasch, A., University of Wisconsin. Madison: Genomic analysis of yeast environmental stress responses.
Johnston, M., University of Colorado School of Medicine, Denver: Feasting, fasting, and fermenting: Glucose sensing by yeasts.
Klar, A., National Cancer Institute, Frederick, Maryland: *Schizosaccharomyces japonicus* yeast poised to become a favorite organism for eukaryotic research and other yeast stories.
Koshland, D., University of California, Berkeley: Stress: From within afar.
Murray, A., Harvard University, Cambridge, Massachusetts: Checkpoint and mitosis/experimental evolution.
Nash, R., Stanford University, Palo Alto, California: Using SGD.
Smith, J., University of Virginia Health System, Charlottesville: Yeast as a model system for aging research.
Toczyski, D., University of California, San Francisco: Identifying ubiquitin ligase substrates.
Winston, F., Harvard Medical School, Boston, Massachusetts: Analysis of transcription and chromatin structure in *S. cerevisiae* and *S. pombe*.

Imaging Structure and Function in the Nervous System

July 24–August 13

INSTRUCTORS
D. DiGregorio, Institut Pasteur, Paris, France
J. Waters, Allen Institute for Brain Sciences, Seattle, Washington
K. Zito, University of California, Davis

ASSISTANTS
G. Moneron, Institut Pasteur, Paris, France
F. Munoz-Cuevas, University of California, San Francisco
W. Oh, University of California, Davis
A. Scimemi, National Institutes of Health, Bethesda, Maryland
K. Smith, Northwestern University, Chicago, Illinois
A. Tran-Van-Minh, Institut Pasteur, Paris, France

Advances in light microscopy and digital image processing and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on basic optics. Students learned the principles of light microscopy, as well as the use of different types of electronic cameras, laser-scanning systems, illumination holographics, 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, and photo-activated ("caged") compounds. Issues raised in imaging with electrophysiological methods were covered. Particular weight was given to multiphoton

laser-scanning microscopy and to genetically encoded fluorophores, especially green fluorescent protein (GFP) and its variants. A spectrum of neural and cell biological systems was used, including living animals and brain slices.

Applicants had a strong background in the neurosciences or in cell biology. In their personal statements, applicants specified (1) their experience with optical techniques, (2) how they will apply optical methods in their current projects, (3) the microscope systems available to them, and (4) their long-term goals in learning more about optical methods.

This course was supported by the Howard Hughes Medical Institute, with additional funding from the Burroughs Wellcome Fund.

PARTICIPANTS

Alvina, K., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Chae, H.G., Ph.D., Cold Spring Harbor Laboratory
Chesler, A., Ph.D., University of California, San Francisco
Chevy, Q., M.S., Pierre & Marie Curie University, Paris, France
Gao, Y., B.S., Pennsylvania State University, State College
Haider, B., Ph.D., UCL Institute of Ophthalmology, London, United Kingdom
Hoffman, E., M.D., Yale University, New Haven, Connecticut
Koukouli, F., M.S., Pasteur Institute Paris France
Lavi, K., B.A., Friedrich Miescher Institute, Basel, Switzerland
Lu, J., Ph.D., Cold Spring Harbor Laboratory
Odstrcil, I., B.A., Harvard University, Cambridge, Massachusetts
Oliveira Martins, A.R., Ph.D., New York University SOM/BEB, New York
Picardo, M., Ph.D., New York University Medical Center, New York
Slezak, M., Ph.D., VIB, Leuven, Belgium

SEMINARS

Albeanu, F., Harvard University, Cambridge, Massachusetts: Intrinsic imaging.
De Grand, A., Olympus America, Inc., Center Valley, Pennsylvania: BX51 optics and objective cleaning.
Deisseroth, K., Stanford University, California: Optogenetics.
Denk, W., Max-Planck-Institute for Medical Research, Heidelberg, Germany: Extended 2P imaging and block-fa.
DiGregorio, D., Institut Pasteur, Paris, France: Point spreads: Intro to lab. Fast Imaging, including VSDs. Practical guide to neurotransmitter uncaging.
DiGregorio, D., Institut Pasteur, Paris, France; Zito, K., University of California, Davis: Flash photolysis.
Dittman, J., Weill Cornell Medical College, New York; Photoactivatable XFPs.
Dombeck, D., Northwestern University, Evanston, Illinois: Awake imaging in hippocampus.
Emiliani, V., CNRS, INSERM, University Paris Descartes, Paris, France: Digital holography.
Engert, F., Harvard University, Cambridge, Massachusetts: Shot noise and intro to noise measurement lab. Two-photon microscopy.
Glickfeld, L., Duke University, Durham, North Carolina: Imaging presynaptic function.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Germany: XFP calcium indicators.
Kilborn, K., Intelligent Imaging Innovations, Inc., Denver, Colorado: Image analysis deconvolution.
Kleinfeld, D., University of California, San Diego, La Jolla: Image analysis ICA, PCA, and denoising. Wavefront aberration, aberration correction.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Basic microscopy.
Lichtman, J., Harvard University, Cambridge Massachusetts: Confocal microscopy.
Mertz, J., Boston University, Massachusetts: Phase-contrast microscopy.
Moneron, G., Institut Pasteur, Paris, France: Super-resolution.
Tsai, P., University of California, San Diego, La Jolla: Scanning and fluorescence. Cameras and PMTs. Saturation. Optics bench lab exercises: Confocal.
Waters, J., Allen Institute for Brain Science, Seattle, Washington: Organic calcium indicators. Homebrew microscope including laser safety.
Weninger, K., North Carolina State University, Raleigh: FRET.
Wilbrecht, L., University of California, San Francisco; Munoz-Cuevas, F., University of California, Emeryville: Image analysis dendrite and spine morphology.

Synthetic Biology

July 30–August 12

INSTRUCTORS
K. Haynes, Arizona State University, Tempe
J. Lucks, Cornell University, Ithaca, New York
D. Savage, University of California, Berkeley
J. Tabor, Rice University, Houston, Texas

ASSISTANTS
R. Davis, Arizona State University, Tempe
D. Nadler, University of California, Berkeley
E. Olson, Rice University, Houston, Texas
M. Takahashi, Cornell University, Ithaca, New York

The goal of synthetic biology is to enable the predictable reprogramming of cells to execute complex physiological activities. It takes inspiration from our ever-expanding ability to measure and perturb biological systems and the philosophical reflections of Schrodinger and Feynman that rational physical laws can be used to describe and engineer biology to accomplish useful things. However, biological systems are noisy, massively interconnected, and nonlinear. The grand challenge for synthetic biology was therefore how to reconcile the desire for a predictable, formalized design process with the inherent "squishiness" of biology.

This course focused on how the complexity of biological systems, combined with traditional engineering approaches, results in the emergence of new design principles for synthetic biology. In the lab, students were introduced to the design–build–test cycle, in which libraries of biological parts are composed into larger modules and evaluated using a variety of high-throughput

techniques. Students worked in teams to solve challenges introduced by the instructors and learned how bacterial and eukaryotic regulation of all forms—transcriptional, translational, post-translational, and epigenetic—can be used to engineer cells to do useful things. In addition, internationally recognized invited speakers gave students a broad overview of applications for synthetic biology, including renewable chemical production and therapeutics, and the current state-of-the-art techniques for both bottom-up and top-down design.

Synthetic biology is an inherently interdisciplinary field. We encouraged students of all backgrounds, whether the very biological or very theoretical, to apply.

This course was equally supported by the U.S. Office of Naval Research and the Howard Hughes Medical Institute.

PARTICIPANTS

Alexander, W., Ph.D., University of Wisconsin, Madison
Al-Khabouri, S., M.Sc., University of Montreal, Canada
Fall, C., Ph.D., Office of Naval Research, Arlington, Virginia
Herd, H., Ph.D., University of Utah, Salt Lake City
Jusiak, B., Ph.D., Baylor College of Medicine, Houston, Texas
Kan, A., M.Phys., University of Cambridge, United Kingdom
MacEachran, D., Ph.D., Logos Technologies, Ashburn, Virginia
Neofotis, P., B.A., City of New York and Brooklyn College, New York
Nygard, Y., M.Sc., VTT Technical Research Centre of Finland, Espoo, Finland
Sander, K., B.S., Bredesen Center Interdiscip., Knoxville, Tennessee
Savitskaya, J., M.Phil., Cambridge University, United Kingdom
Singhal, V., B.Eng., California Institute of Technology, Pasadena
Spring, K., Ph.D., University of Texas Health Science Center, Houston
Vogl, T., M.Sc., Graz University of Technology, Austria
Ward, J., Ph.D., Middlebury College, Middlebury, Vermont
Wells, D., B.A., Northwestern University, Evanston, Illinois

SEMINARS

Arkin, A., University of California, Berkeley: Context effects and predictability in gene expression.
Chang, M., University of California, Berkeley: Engineering microbes for chemical synthesis.
Ellington, A., University of Texas, Austin: Synthetic biology: An alternative view.
Gallivan, J., Emory University, Atlanta, Georgia: Reprogramming bacteria with small molecules and RNA.
Gibson, D., J. Craig Venter Institute, San Diego, California: Building a synthetic cell and advancing synthetic genomics.
Jewett, M., Northwestern University, Evanston, Illinois: Establishing cell-free synthetic biology for materials.
Klavins, E., University of Washington, Seattle: Control of cellular state and decision making in synthetic.
Murray, R., California Institute of Technology, Pasadena: Biomolecular breadboards for prototyping and debugging.
Palmer, M., University of California, Berkeley: Practicing synthetic biology: (How) can we be responsible?
Silver, P., Harvard Medical School, Boston, Massachusetts: How to think about designing biological systems.
Wang, H., Columbia University, New York: Multiplexed genome engineering methods and applications.
Weiss, R., Massachusetts Institute of Technology, Cambridge: From parts to modules to therapeutic systems in mammalian.

Cellular Biology of Addiction

August 6–12

INSTRUCTORS
A. Bonci, National Institute on Drug Abuse, Baltimore, Maryland
C. Evans, University of California, Los Angeles
B. Kieffer, IGBMC, Cedex, France
R. Maldonado, Pompeu Fabra University, Barcelona, Spain
M. Von Zastrow, University of California, San Francisco

ASSISTANTS
P. Chu Sin Chung, IGBMC, Cedex, France
A. Taylor, University of California, Los Angeles

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 the proposed workshop was to provide an intense dialog of the fundamentals, state-of-the-art advances, and major gaps in the cell and molecular biology of drug addiction. Targeted to new or experienced investigators, the workshop combined formal presentations and informal discussions to convey the merits and excitement of cellular and molecular approaches to drug addiction research. With the advent of genomics and proteomics, an extraordinary opportunity now exists to develop comprehensive models of 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; neuroadaptive processes at the molecular and cellular level; neural networks and their modulation; the relevance of genotype to susceptibility and drug response; tolerance and adaptation at the cellular level; and approaches to exploiting the daunting volume generated by neuroinformatics. This

workshop provided an integrated view of current and novel research on neuroadaptive responses to addiction, fostered discussion on collaboration and integration, provided critical information needed to construct a model of addiction as a disease and novel molecular targets for biological treatments. Beyond the plane of scientific endeavor, the information is vital for formulating public policy and for enlightening the public on the neurobiological consequences of drug use and addiction. The workshop was designed to generate interest in this level of analysis, open conduits for collaborations, and present novel routes to investigating the neurobiology of addictive drugs.

This course was supported with funds provided by the National Institute on Drug Abuse.

PARTICIPANTS

Ables, J., Ph.D., The Rockefeller University, New York
Abraham, A., Ph.D., Oregon Health & Science University, Portland
Aydin, C., Ph.D., University of Michigan, Ann Arbor
Banghart, M., Ph.D., Harvard Medical School, Boston, Massachusetts
Barr, J., Ph.D., Temple University, Philadelphia, Pennsylvania
Bocarsly, M., B.A., Princeton University, New Jersey
Brown, J., Ph.D., University of Missouri, Columbia
Brown, R., Ph.D., Medical University of South Carolina, Charleston
Damez Werno, D., M.S., Icahn School of Medicine at Mount Sinai, New York
Frohmader, K., Ph.D., University of Michigan, Ann Arbor
Graham, D., B.S., Vanderbilt University Medical Center, Nashville, Tennessee
Hancock, D., B.S., RTI International, Research Triangle Park, North Carolina
Ko, J., Ph.D., Seton Hall University, South Orange, New Jersey
McIver, S., Ph.D., Tufts University, Boston, Massachusetts
Orsini, C., Ph.D., University of Florida, Gainesville
Panarsky, R., Ph.D., NIAAA/LNG, National Institutes of Health, Rockville, Maryland
Parker, R., Ph.D., California Institute of Technology, Pasadena
Pick, J., Ph.D., New York University Medical Center, New York
Ramaker, M., B.S., Oregon Health & Science University, Portland
Spencer, S., Ph.D., Medical University of South Carolina, Charleston
Yeo, S., Ph.D., National Institute on Alcohol Abuse and Alcoholism, Bethesda, Maryland

SEMINARS

Belin, D., INSERM, Lyon, France: Corticostriatal substrates of incentive habits*:* Relevance for the pathophysiology of addictions.
Bonci, National Institute on Drug Abuse/NIDA, Rockville, Maryland: Optogenetics and synaptic plasticity: Implications of substance abuse.
Chavkin, C., University of Washington, Seattle: Understanding stress as a risk factor for addiction.
Edwards, R., University of California, San Francisco: The corelease of glutamate with monoamines.
Goldman, D., NIAAA/LNG, National Institutes of Health, Rockville, Maryland: Genetics of addiction: Alleles, mechanisms, and contexts.
Kalivas, P., Medical University of South Carolina, Charleston: Curing addiction with synaptic plasticity.
Kenny, P., The Scripps Research Institute, La Jolla, California: Mechanisms by which hypocretin (orexin) regulates drug intake.
Kieffer, B., IGBMC, Cedex, France: Opioid receptor function in addiction and mood disorders: Mouse genetic approaches.
Koob, G., The Scripps Research Institute, La Jolla, California: Neurobiology of addiction: View from the dark side.
Mason, B., The Scripps Research Institute, La Jolla, California: Predictive validity of human laboratory models for medication development in alcohol dependence.
Nairn, A., Yale University School of Medicine, New Haven, Connecticut: Signal transduction mechanisms beyond the dopamine receptor.
Nestler, E., Mount Sinai University, New York: Transcriptional and epigenetic mechanisms of addiction.
Picciotto, M., Yale University, New Haven, Connecticut: Molecular basis of nicotine addiction.
Pierce, C., University of Pennsylvania, Philadelphia: Forgiving the sins of the father: Epigenetic inheritance of addiction phenotypes.
Pollock, J., National Institute on Drug Abuse/NIH/DHHS, Rockville, Maryland: The genetics program at NIDA.
Sinha, R., Yale University, New Haven, Connecticut: Clinical neurobiology of stress and addiction.
Volkow, N., National Institutes of Drug Addiction, Rockville, Maryland: Progress and priorities in drug abuse and addiction research.
Yang, W., University of California, Los Angeles: Genetic dissection of basal ganglia circuitry: Novel mechanistic insights into opiate reward.

X-Ray Methods in Structural Biology

October 14–29

INSTRUCTORS **W. Furey,** V.A. Medical Center/University of Pittsburgh, Pennsylvania
G. Gilliland, Janssen R&D, LLC/Johnson & Johnson, Radnor, Pennsylvania
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku Americas Corporation, The Woodlands, Texas

ASSISTANT **M.J. Whitley,** University of Pittsburgh, Pennsylvania

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensified laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics 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, crystal twinnings, 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 Institute of General Medical Sciences.

PARTICIPANTS

Barajas, J., B.S., University of California, Irvine
Benning, F., B.Sc., University of Basel, Switzerland
Chen, P.-H., B.S., Northwestern University Feinberg School of Medicine, Chicago, Illinois
Galicia, C., B.S., Instituto de Quimica, UNAM, Mexico
Griffith, E., Pharm., St. Jude Children's Research Hospital, Memphis, Tennessee
Jackson, R., Ph.D., Montana State University, Bozeman
Karr, E., Ph.D., University of Oklahoma, Norman
Kuiper, E., B.S., Emory University, Atlanta, Georgia
Lansky, S., B.S., The Hebrew University of Jerusalem, Israel
Meekins, D., B.S., University of Kentucky, Lexington
Michel, S., Ph.D., University of Maryland School of Pharmacy, Baltimore
Musselman, C., Ph.D., University of Iowa, Iowa City
Ragland, D., B.S., University of Massachusetts Medical School, Worcester
Ron, D., M.D., University of Cambridge, United Kingdom
Vasiliauskaite, I., B.S., The Pasteur Institute, Paris, France
Wu, P., M.S., Genentech, Inc., S. San Francisco, California

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, California: Structure refinement. PHENIX overview.
Caffrey, M., Trinity College Dublin, Ireland: Crystallizing membrane proteins for structure-function studies using lipidic mesophases.
Calero, G., University of Pittsburgh School of Medicine, Pennsylvania: Nano crystals and nano seeds.
Emsley, P., University of Glasgow, Oxford, United Kingdom: Model-building tools in coot.
Furey, W., V.A. Medical Center/University of Pittsburgh, Pennsylvania: Patterson group therapy. Anomalous data collection considerations. Isomorphous replacement and anomalous scattering. Solvent flattening/phase combination. MAD phasing: A classical approach. The role of direct methods in macromolecular crystallography. Noncrystallographic symmetry averaging.
Gilliland, G., Janssen R&D, LLC/Johnson & Johnson, Radnor, Pennsylvania: Course overview: The structure determination of biological macromolecules. Maximizing crystallization success through seeding.
Hendrickson, W., Columbia University, New York: MAD and SAD phasing.
Kleywegt, G., European Bioinformatics Institute, Cambridge, United Kingdom: 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. The art of capillary crystal mounting. Heavy atoms and anomalous scatters.
Perrakis, A., Netherlands Cancer Institute, Amsterdam, The Netherlands: Automated model building and rebuilding: From ARP/WARP to PDB_REDO. Macromolecular animation.
Pflugrath, J., Rigaku Americas Corporation, The Woodlands, Texas: Data collection: Design and setup. Cryocrystallography. 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: Headd, J., Lawrence Berkeley Laboratory, California: Detection and repair of model errors using MolProbity.
Richardson, J., Duke University Medical Center, Durham, North Carolina: Structure presentation.
Smith, C., Stanford University, California: Synchrotron data collection and femtosecond crystallography.
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.
Thorn, A., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: An introduction to SHELXC/D/E. The basics of twinning in crystals of macromolecules.
Tronrud, D., Oregon State University, Corvalis: Macromolecular refinement I. Macromolecular refinement II. Electron density maps.
Westbrook, J., Rutgers University, Piscataway, New Jersey: Automating PDB deposition.

Programming for Biology

October 14–29

INSTRUCTOR **S. Prochnik,** DOE–Joint Genome Institute, Walnut Creek, California

CO-INSTRUCTOR **S. Robb,** University of California, Riverside

ASSISTANTS **S. Ahrendt,** University of California, Riverside
J. Bredeson, University of California, Berkeley
D. Messina, Cofactor Genomics, St. Louis, Missouri
E. Ross, Howard Hughes Medical Institute, Kansas City, Missouri
D. Triant, University of Virginia, Charlottesville

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 is generated. Designed for students and researchers with little or no prior programming experience, this 2-week course gave biologists the bioinformatics skills necessary to exploit this abundance of biological data.

The course was based around the Perl scripting language because of its ease of learning and incredible wealth of ready-built modules, such as Bioperl, that was designed to solve common

biological problems. Starting with introductory coding and continuing with a survey of available biological libraries and practical topics in bioinformatics, students ended by learning how to construct and run powerful and extensible analysis pipelines in a straightforward manner. The course combined formal lectures with hands-on sessions in which students work 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 each other and the faculty to solve them. Final projects have formed the basis of publications as well as public biological websites.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Adeogun, S., B.S., Fisk University, Nashville, Tennessee
Ameziane, N., Ph.D., Vrije Universitet Medical Center, Amsterdam, The Netherlands
Blaby, I., Ph.D., University of California, Los Angeles
Boyle, J., B.S., Harvard University, Cambridge, Massachusetts
Chevrier, N., Ph.D., Harvard University, Cambridge, Massachusetts
Clark, V., B.A., Yale University School of Medicine, New Haven, Connecticut
Duggan, A., M.S., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Franke, K., Ph.D., University of Delaware, Newark
Gallaher, S., Ph.D., University of California, Los Angeles
Glover-Cutter, K., B.Sc., U.S. Department of Agriculture, Corvallis
Goldmann, J., Ph.D., Whitehead Institute/MIT, Cambridge, Massachusetts
Guo, L., B.S., Stowers Institute for Medical Research, Kansas City, Missouri
Jones, K., B.Sc., University of Waterloo, Canada
Kantar, M., Ph.D., University of Minnesota, St. Paul
Keasey, S., B.S., U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland
Landau, D., M.D., Dana-Farber Cancer Institute, Boston, Massachusetts
MacHugh, D., Ph.D., University College of Dublin, Ireland
Malone, C., Ph.D., New York University Medical School, Skirball Institute, New York
McDevitt, S., B.A., Children's Hospital Oakland Research Institute, Oakland, California
Paul, A., Ph.D., Cold Spring Harbor Laboratory
Rehan, S., Ph.D., University of Pennsylvania, Philadelphia
Ruiz, N., M.S., University of Utah, Salt Lake City
Turner, J., Ph.D., University of Pennsylvania, Philadelphia
Wang, H.-L., B.S., University of Missouri, Kansas City

SEMINARS

Cain, S., Ontario Institute for Cancer Research, Medina, OH: GBrowse.
Haas, B., Broad Institute, Cambridge, Massachusetts: RNA-Seq expression analysis. RNA-Seq expression. HTML.
Marques-Bonet, T., University of Pompeu Fabra/CSIC, Barcelona, Spain: Structural variant analysis.
Moore, B., University of Utah, Salt Lake City: Genome annotation with MAKER. VAAST variant analysis pipeline.
Pearson, W., University of Virginia, Charlottesville: Sequence similarity searches.
Prochnik, S., DOE–Joint Genome Institute, Walnut Creek, California: CGI.
Robb, S., University of California, Riverside: Bioperl.
Schatz, M., Cold Spring Harbor Laboratory: Genome assembly with ALLPATHS–LG.
Stajich, J., University of California, Riverside: Introduction to next-generation sequencing and SNP calling.
Tisdall, J., Baltimore, Maryland: Scientific computing.

Computational and Comparative Genomics

November 6–12

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 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 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.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Auble, D., Ph.D., University of Virginia Health System, Charlottesville
Baldridge, D., M.D., Ph.D., St. Louis Children's Hospital, Missouri
Brukhin, V., Ph.D., St. Petersburg State University, Russia
Buckner, B., Ph.D., Truman State University, Kirksville, Missouri
Carey, W., Ph.D., Intertrust Corp., Sunnyvale, California
Doss, J., Ph.D., Duke University, Durham, North Carolina
Dupont, P.-Y., Ph.D., Massey University, Palmerston North, New Zealand
Elgin, S., Ph.D., Washington University, St. Louis, Missouri
Guler, G., Ph.D., Genentech, S. San Francisco, California
Hapala, J., M.Sc., Masaryk University, Brno, Czech Republic
Hopper, K., Ph.D., USDA-Agricultural Research Service, Newark, Delaware
Kozel, B., M.D., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Miller, D., B.A., Stowers Institute for Medical Research, Kansas City, Missouri
Mitchell, D., Ph.D., Texas Tech University Health Sciences Center, El Paso
Palmer, S., Ph.D., University of Florida, Gainesville
Paudel, Y., M.S., Wageningen University, Wageningen, The Netherlands
Renn, S., Ph.D., Reed College, Portland, Oregon
Theisen, J., M.D., Ph.D., St. Louis Children's Hospital, St. Louis, Missouri
Ziegler, K., B.Sc., University of Alberta, Edmonton, Canada

SEMINARS

Mackey, A., University of Virginia, Charlottesville: Genome annotation I (HMM basics). Annotation review Yandell. Sequencing technologies/tools. SNP discovery and variation. RNA-Seq. From gene lists to pathways.
Mills, L., University of Virginia, Charlottesville: The UCSC genome browser. Open helix UCSC browser intro UCSC exercises. Review/Panel Discussion–Ensembl/UCSC–which browser when?
Overduin, B., EMBL–European Bioinformatics Institute, Hinxton, Cambridgeshire, United Kingdom: The Ensembl database of genomes I. Ensembl/BioMart; BioMart/API Demos. Ensembl API.
Pearson, W., University of Virginia, Charlottesville; Stubbs, L., University of Illinois, Urbana: Introduction and overview. Protein evolution and sequence similarity sequencing. Practical sequence similarity searching. Alignment algorithms and scoring matrices. PSSMs, HMMs, and Pfam. Multiple sequence alignment and phenotype prediction. PolyPhen/SIFT.
Stormo, G., Washington University School of Medicine, St. Louis, Missouri: Modeling motifs: Collecting data. From motifs to regulatory networks.
Stubbs, L, University of Illinois, Urbana: Introduction to genome biology. Using the ECR browser. zPicture/MULAN chromosome alignment. MEME motif funding. Cisfinder. DAVID exercise. DAVID gene list. DAVID paper. Integrating genomics data sets for biological inference. Comparative epigenomics demo. Epigenome browser from UCSC. WashU epigenome browser.
Taylor, J., Emory University, Atlanta, Georgia: Galaxy for high-throughput analysis.

Antibody, Engineering, and Phage Display

November 6–19

INSTRUCTORS
C. Barbas, Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, New York University School of Medicine, New York

ASSISTANTS
S. Gilgunn, Dublin City University, Ireland
S. Kacir, University of Pennsylvania, Philadelphia
M.A. Pohl, Albert Einstein College of Medicine, Bronx, New York

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of antibody fragments expressed in *E. coli* were also covered. Epitopes were selected from peptide libraries and characterized.

The lecture series, presented by the course instructors and invited speakers, emphasized antibody structure and function, alternative display technologies and scaffolds, the immunobiology of the antibody response, and the use of antibodies and antibody conjugates for therapy.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Aguilar, J., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Bethke, A., Ph.D., Cornell University, Ithaca, New York
Chirichella, M., M.S., Scuola Normale Superiore, Pisa, Italy
Dobrenkov, K., M.D., Memorial Sloan-Kettering Cancer Center, New York
Fleetwood, F., B.S., Royal Institute of Technology, Stockholm, Sweden
Fofana, I., Ph.D., Boston College, Chestnut Hill, Massachusetts
Hatahet, F., B.Sc., Harvard Medical School, Boston, Massachusetts
Heinen, R., M.D., Institute of Molecular Biology, Vienna, Austria
Juncker, D., Diplom., McGill University, Montreal, Canada
Kanter, J., Ph.D., Medimmune, Frederick, Maryland
Lin, C.-W., M.S., Academia Sinica, Taipei, Japan
Lopez Albaitero, A., M.D., Memorial Sloan-Kettering Cancer Center, New York
Mercan, F., Ph.D., Cold Spring Harbor Laboratory
Roberts, S., B.S., Duke University, Durham, North Carolina
Viadiu, H., Ph.D., University of California, San Diego, La Jolla
Vuong, C., B.S., Texas A&M University, College Station

SEMINARS

Boyd, S., Stanford University, California: High-throughput sequencing of human antibody repertoires.
Crowe, Jr., J., Vanderbilt University Medical Center, Nashville, Tennessee: Integrated human B-cell repertoire studies using hybridoma and next-generation sequence analysis.
Noren, C.; Noreen, K., New York England Biolabs, Beverly, Massachusetts: Phage peptide libraries: The PhD for peptides.
Rader, C., The Scripps Research Institute-Florida, Jupiter, Florida: Antibody engineering and conjugation technologies for cancer therapy.
Siegel, D., University of Pennsylvania Medical Center, Philadelphia: Human phage display libraries, cell surface panning, and translational medicine applications.
Silverman, G.J., New York University Medical Center, New York: Superantigens and immune repertoires.
Stahl, S., AlbaNova University Center, Stockholm, Sweden: Selecting affibody molecules for applications in cancer and Alzheimer's disease.
Wilson, I., The Scripps Research Institute, La Jolla, California: Broadly neutralizing antibodies to HIV and influenza viruses.

Advanced Sequencing Technologies and Applications

November 12–24

INSTRUCTORS

E. Mardis, Washington University School of Medicine, St. Louis, Missouri
G. Marth, Boston College, Chestnut Hill, Massachusetts
W. 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
E. Garrison, Boston College, Chestnut Hill, Massachusetts
E. Ghiban, Cold Spring Harbor Laboratory
S. Goodwin, Cold Spring Harbor Laboratory
M. Griffith, Washington University School of Medicine, St. Louis, Missouri
O. Griffith, Washington University School of Medicine, St. Louis, Missouri
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
S. McGrath, Washington University School of Medicine, St. Louis, Missouri
C. Miller, Boston College, Chestnut Hill, Massachusetts
J. Walker, Washington University School of Medicine, St. Louis, Missouri
A. Ward, Boston College, Chestnut Hill, Massachusetts

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, enabling 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 studying small RNAs, among others. Guest lecturers highlighted their own applications of these revolutionary technologies.

We encouraged applicants from a diversity of scientific backgrounds including molecular evolution, development, neuroscience, cancer, plant biology, and microbiology.

This course was supported by the National Human Genome Research Institute, with major equipment provided by Illumina & Life Technologies.

PARTICIPANTS

Allam, B., Ph.D., Stony Brook University, Stony Brook, NY
Apparsundaram, A., Ph.D., HySynth Biotechnologies, North Caldwell, NJ
Beeler, J.S., Ph.D., Vanderbilt University School of Medicine, Nashville, TN
Coban Akdemir, Z., Ph.D., M.D. Anderson Cancer Center, Houston, TX
Dermawan, K.T., Ph.D., Cleveland Clinic Lerner Research Institute, Ohio
Engle, E., Ph.D., Washington University, St. Louis, Missouri
Espitia, H., M.Sc., Colombian Sugarcane Research Center, Cali, Colombia
Goldlust, I., Ph.D., National Institutes of Health, Rockville, Maryland
Hapala, J., M.Sc., Masaryk University, Brno, Czech Republic
Higgins, G., Ph.D., Mayo Clinic, Assure Rx Health, Inc., Mason, Ohio
Jeukens, J., Ph.D., University Laval, Quebec, Canada
Masamha, C., Ph.D., University of Texas Health Science Center, Houston
Melas, P., Ph.D., Karolinska Institute, Stockholm, Sweden
O' Brien, E., Ph.D., University of Cambridge, United Kingdom
Oh, Y.-S., Ph.D., The Rockefeller University, New York
Oppenheim, S., Ph.D., American Museum of Natural History, New York
Schafer, M., Ph.D., New York University, New York
Stadler, Z., M.D., Memorial Sloan-Kettering Cancer Center, New York
Tihon, E., Ph.D., Institute of Tropical Medicine, Antwerp, Belgium
Uyar, A., Ph.D., Yale School of Medicine, New Haven, Connecticut

SEMINARS

Baslan, T., Cold Spring Harbor Laboratory: Single cancer cell sequencing.
Dewar, K., McGill University and Genome QC Innovation Centre, Montreal, Canada: Clinical sequencing of *Clostridium difficile* isolates with Illumina and PacBio hybrid assembly.
Griffith, M., Washington University School of Medicine, St. Louis, Missouri: RNA-Seq introduction.
Hall, I., University of Virginia, Charlottesville: Background talk: Structural variation prevalence, mechanisms, biological impact. Structural variant detection and cancer-specific aspects. Practical exercise on structural variant detection.
Hodges, E., Cold Spring Harbor Laboratory; Methylation and ChIP-Seq: Methods and analysis.
Kramer, M., Cold Spring Harbor Laboratory: Illumina PE and ion torrent read processing, base-calling pipeline, interpreting results.
Magrini, V.; McGrath, S., Washington University School of Medicine, St. Louis, Missouri: Overview of next-generation sequencing technologies and sample prep methods.
Mardis, M., Washington University School of Medicine, St. Louis, Missouri: NGS in cancer genomics studies.
Marth, G., Boston College, Chestnut Hill, Massachusetts; SNP callers and other downstream analytical processing.
Quinlan, A., University of Virginia, Charlottesville; Yandell, M., University of Utah, Salt Lake City: Variant interpretation: Interactive interrogation of variants with Aaron's GEMINI, and disease gene hunting with Mark Yandell's VAAST.

Schatz, M., Cold Spring Harbor Laboratory: Hybrid assembly of NGS data.

Walker, J.; Griffith, O.; Griffith, M., Washington University School of Medicine, St. Louis, Missouri: Introduction to Unix commands and file system organization. Continue Unix commands, BASH, Perl, R, etc. RNA-Seq alignment and visualization. RNA-Seq expression and differential expression. RNA-Seq isoform discovery and alternative expression. RNA-Seq finale.

Walker, J.; Griffith, O., Washington University School of Medicine, St. Louis, Missouri: RNA-Seq tuxedo suite.

Zody, M., Broad Institute, Cambridge, Massachusetts: Introduction to IGV viewer. RNA-Seq de novo assembly.

The Genome Access Course

The Genome Access Course (TGAC) is an intensive 2-day introduction to bioinformatics that was held three times in 2013 and trained more than 110 participants in total. Registration for the course is open to all on a first-come, first-served basis subject to basic eligibility requirements. The core of TGAC covers the manipulation and analysis of sequence data using publicly available tools. The course is broken into modules designed to give a broad overview of a given topic, with ample time for hands-on exercises chosen by the instructors. Each module includes three parts: (1) a discussion of theory and methods, (2) coverage of software and Web resources, and (3) use of selected tools in practice. The modular design allows the instructors to tailor the curriculum to the interests of the students. Modules in 2013 included Genome Sequencing and Assembly, Gene Prediction, the UCSC Genome Browser, Ensembl, Comparative Genome Analysis, Gene Set Enrichment and Pathway Analysis, High-Throughput Sequence Analysis, the Galaxy Project, and RNA-Seq Analysis Using R. Students were encouraged to bring questions and data from their own research projects to the course, and they were also encouraged to contact instructors with additional questions once they returned to their home institutions.

In April and July, TGAC was held in CSHL's newly renovated Hershey Laboratory, which opened in the summer of 2012.

INSTRUCTORS

C. Lambert, Cold Spring Harbor Laboratory
G. Howell, The Jackson Laboratory
B. King, Mount Desert Island Biological Laboratories
J. Ward, Middlebury College

LECTURERS

A. Gordon, Cold Spring Harbor Laboratory
E. Hodges, Cold Spring Harbor Laboratory
M. Schatz, Cold Spring Harbor Laboratory

April 21–23

Students: 30

PARTICIPANTS

Bacanamwo, M., Morehouse School of Medicine
Carmona, M., Yale University
Ciarleglio, C., Brown University
D'Alessandro, L., The Hospital for Sick Children
Diaz, P., University of Connecticut Health Center
Guallar, D., Mount Sinai School of Medicine
Higgins, G., Mayo Clinic; AssureRx Health, Inc.
Higgins, M., Roswell Park Cancer Institute
Janha, R., Medical Research Council Unit The Gambia
Jin, K., University of Medicine & Dentistry of New Jersey
Kasmar, A., Harvard Medical School
Leda, A. R., The Aaron Diamond AIDS Research Center
Lucs, A., Feinstein Institute for Medical Research
Marsh, J., University of Pittsburgh
Moss, W., Yale University/HHMI
Mustapha, M., University of Pittsburgh
Newsome, C., Child Health Institute of New Jersey (UMDNJ/RWJMS)
Novatt, J., Cold Spring Harbor Laboratory
O'Rawe, J., Cold Spring Harbor Laboratory
Schneyer, A., University of Massachusetts, Amherst
Seth, R., Yale School of Medicine
Shakur, R., University of Cambridge
Stansell, E., Harvard Medical School/NEPRC
Tian, Y., University of Pennsylvania
Wang, C.-L., Cold Spring Harbor Laboratory
Wang, J., Mount Sinai School of Medicine
Watson, P., Memorial Sloan-Kettering Cancer Center
Wong, J., Mount Sinai School of Medicine
Zuber, M., SUNY Upstate Medical University
Zukher, I., Institute of Gene Biology

July 18–20

Students: 35

PARTICIPANTS

Klingener, M., Cold Spring Harbor Laboratory
Knowlton, D., RaNA Therapeutics
Lo, J., Harvard/MGH
Maze, I., The Rockefeller University
Nemeth, K., University of Minnesota Medical School, Duluth
Pena, C., Mount Sinai School of Medicine
Reshmi, S., Nationwide Children's Hospital
Rezza, A., Black Family Stem Cell Institute. Mount Sinai
Ruan, T., Stony Brook University
Santiago, J., Rosalind Franklin University
Schnyder, S., Boston Medical Center
Sennett, R., Mount Sinai School of Medicine
Simien, C., University of Texas M.D. Anderson Cancer Center
Sintov, E., Tel-Aviv University
Sokolow, S., University of California, Los Angeles
Stundon, J., Princeton University
Todd, J., Children's Hospital Boston
Toussaint, V., Agriculture and Agri-Food Canada
Turner, J., University of Pennsylvania
Veronneau, P.-Y.,
Villani, S., Cornell University
Walker, D., Mount Sinai School of Medicine
Westby, G., Memorial Sloan-Kettering Cancer Center
Zhan, H., James J. Peters VA Medical Center
Zhang, Y., Stony Brook University
Zuo, L., Yale University

The November course was held in Manhattan at the brand-new facilities of the New York Genome Center, and it marked the first collaborative training project with the New York Genome Center, for which CSHL is an institutional founding member. The November course had a record high enrollment of 48 students with more than 20 on a waiting list, a majority of who were from institutions in the New York City area. CSHL plans to offer the Manhattan course again in July 2014 in collaboration with the New York Genome Center, and annually thereafter as long as demand for the course continues.

November 17–19

Students: 48

INSTRUCTORS
G. Howell, The Jackson Laboratory
B. King, Mount Desert Island Biological Laboratories
C. Lambert, Cold Spring Harbor Laboratory
B. Overduin, EMBL-European Bioinformatics Institute
J. Ward, Middlebury College

LECTURERS
D. Church, DHHS/NIH/NLM/NCBI
E. Hodges, Cold Spring Harbor Laboratory

PARTICIPANTS

Aggarwal, A., Mount Sinai School of Medicine
Alshafai, K., Imperial College of London
Banaszynski, L., The Rockefeller University
Barton, G., University of California, Berkeley
Benazet, J.-D., Weill Cornell Medical College
Carmona-Fontaine, C., Memorial Sloan-Kettering Cancer Center
Cattani, V., New York University
Cerdeira, L.T., Fleury, South America
Chheda, M., Memorial Sloan-Kettering Cancer Center
Coux, R., New York University School of Medicine/Skirball Institute
D'Angelo, C.S., University of Sao Paulo
Fariello, D., Fordham University
Garabedian, M., New York University School of Medicine
Griffiths, B., University of Massachusetts
Hafner, M., The Rockefeller University
Hsu, Y.-C., The Rockefeller University
Huebner, M., Cold Spring Harbor Laboratory
Jain, A., Systems Biology Center, New York
Jarvis, J., University at Buffalo
Knapp, R., University of Oklahoma
Kutluay, S., Aaron Diamond AIDS Research Center
Loughran. O., Virginia Commonwealth University
Matov, A., Weill Cornell Medical College
Miura, P., Memorial Sloan-Kettering Cancer Center
Moore, M., Stony Brook University
Pande, P., Boehringer Ingelheim Pharmaceuticals
Park, H.Y., Albert Einstein College of Medicine
Patel, J., Janssen Pharmaceuticals (Johnson & Johnson)

Pavlicev, M., Cincinnati Children's Hospital
Pelton, S., Boston University School of Medicine
Petros, T., New York University
Rosa Fernandes, L., Fundação Faculdade de Medicina
Sabari, B., The Rockefeller University
Sabharwal, V., Boston University Medical Center
Sanfilippo, P., Gerstner Sloan-Kettering Graduate School of Biomed
Schneiderman, J., Memorial Sloan-Kettering Cancer Center
Sheng, Q., Novartis
Sohn, J., Yonsei Cancer Center
Tahtinen, P., Boston University
Tang, L., Memorial Sloan-Kettering Cancer Center
Veerappan, R., SiParadigm Diagnostics Informatics
Wamsley, B., New York University Langone Medical Center
Will, B., Albert Einstein College of Medicine
Wontakal, S., Columbia University
Xu, W., Synthetic Genomics, Inc.
Yazbeck, V., Massey Cancer Center
Yu, L., The Ohio State University
Zhao, Y.,(Jeffrey), Memorial Sloan-Kettering Cancer Center

The Laboratory acknowledges the generosity of the following companies that loaned equipment and supplied free or discounted reagents to the various courses:

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Kopf Instruments
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Tecan US, Inc.
Thermo Fisher Scientific
Thor Labs
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Warner Instruments
Waters Corporation
World Precision Instruments

SEMINARS

INVITED SPEAKERS PROGRAM ("CSHL SEMINAR SERIES")

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, providing an opportunity for the exchange of ideas in an informal setting.

	Title	Host
January		
James Eberwine, University of Pennsylvania	Single-cell mRNA variation—Why?	Adrian Krainer
Tim Stearns, Stanford University School of Medicine	Centrosomes, cilia, and cell signaling.	Bruce Stillman
Nadrian Seeman. New York University	DNA is not merely the secret of life: Using molecular information for the control of 3D structure.	Leemor Joshua-Tor
February		
Anne Brunet, Stanford University School of Medicine	Epigenetic regulation of aging.	Lloyd Trotman
Iannis Aifantis, New York University School of Medicine	Regulation of cancer stem cell function by the ubiquitin/proteasome system.	Scott Powers
Michael Rosbash, Brandeis University	Genome-wide approaches provide new insights into circadian regulation.	Christopher Hammell
Carol Prives, Columbia University	Lessons learned from studying the p53 network.	Terri Grodzicker
March		
John Lis, Cornell University	New views of transcription and its regulation.	Christopher Hammell
Robert Singer, Albert Einstein College of Medicine of Yeshiva University	Following single mRNAs in living cells and tissues.	David Spector
April		
Yang Shi, Harvard Medical School	A histone methylation network regulates transgenerational epigenetic inheritance in *C. elegans.*	Hongwu Zheng
Miguel Nicolelis, Duke University Medical Center	Principles of neural ensemble physiology and brain–machine interfaces.	Hassana Oyibo
October		
Stuart Schreiber, Broad Institute of MIT and Harvard	Toward a chemistry-enabled patient-based drug discovery.	Scott Powers
Jean Zhao, Dana Farber Cancer Institute	P13 K isoforms in signaling and cancer: Mechanistic and therapeutic insights from GEM models.	Lloyd Trotman
November		
Dana Pe'er, Columbia University	On the road to personalized therapy: Dealing with heterogeneity within and between tumors.	Scott Powers
Brian Shoichet, University of Toronto	The metabolic code.	Leemor Joshua-Tor
Sean Morrison, University of Texas Southwestern Medical Center	Stem cell self-renewal and leukemogenesis.	Grigori Enikolopov
December		
Takao Hensch, Harvard University	Shaping neural circuits by early experience.	Stephen Shea
Elaine Fuchs, The Rockefeller University	Skin stem cells in silence, action and cancer.	Terri Grodzicker

IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor Laboratory's In-House Seminars were initiated to provide a semiformal venue for communication among the various research groups at the Laboratory. The seminars also afford a necessary opportunity for graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

Speaker	Title
January	
Jesse Gillis	Network-based interpretation of gene function from postsynaptic protein interaction to schizophrenia coexpression.
Greg Hannon	A small RNA-based innate immune system guards germ cell genomes.
Mikala Egeblad	Innate immune cells as villains and heroes in the host response to cancer.
James D. Watson	Oxidants, antioxidants, and the current incurability of metastatic cancers.
February	
Bo Li	Brain mechanisms of fear in health and disease.
Wangzhi Li (Mills Lab)	A gift package from Dad: Chd5 orchestrates rewrapping of the sperm genome.
March	
Jim Hicks	Where's Waldo? Can rare circulating tumor cells help direct cancer treatment in real time?
Hongwu Zheng	Understanding malignant glioma pathogenesis.
Shane McCarthy (McCombie Lab)	Harvesting rare variation in schizophrenia.
Junwei Shi (Vakoc Lab)	Cooperation between chromatin readers and remodelers in the pathogenesis of cancer.
April	
Martin Akerman (Krainer Lab)	A deep dive into high-throughput data: Analysis of alternative splicing using RNA-Seq.
Anne Churchland	Optimal weighting of sensory information for decision-making.
Andrea Eveland (Jackson Lab)	Defining developmental mechanisms in maize using an integrative genomics approach.
October	
Michael Schatz	Algorithms for the analysis of complex genomes.
Duda Kvitsiani (Kepecs Lab)	Foraging decisions revealed by cell-type-specific recordings in anterior cingulate cortex.
November	
Qiaojie Xiong (Zador Lab)	Corticostriatal synaptic weights encode arbitrary associations between stimuli and motor responses.
Simon Knott (Hannon Lab)	Applying functional genomics to a mouse model of tumor heterogeneity and metastasis.
Michael Huebner (Spector Lab)	The role of the histone demethylase JMJD3/KDM6B in transcriptional induction.
Grisha Enikolopov	Stem cells of the adult brain.
December	
Glenn Turner	Input-output transformation in the mushroom body.

BANBURY CENTER

BANBURY CENTER
EXECUTIVE DIRECTOR'S REPORT

It was just over a year ago that our region was devastated by Superstorm Sandy, and I am glad to say that there is little, if any, evidence left of its impact on Banbury. The Robertson House roof was speedily repaired, the debris cleared up, and the fallen trees reduced to chips. The weather continued to be interesting with a major snowstorm in February, 2013, but fortunately Banbury was occupied by the Watson School of Biological Sciences' course on microbial pathogenesis. The participants in the course did not have far to travel to get here.

One lingering effect of the storm was that meetings which were to have been held in 2012 were moved to 2013, so that the year was rather busy. We held 23 meetings with more than 700 participants, in addition to three Watson School courses and six Cold Spring Harbor Laboratory courses. Altogether, the Center was used on 37 occasions in 2013. Participants in the meetings were drawn from no fewer than 40 states, probably a record, although, as usual, four states—California, Maryland, Massachusetts, and New York—accounted for 51% of participants. Twenty-nine percent of participants were female, a proportion that has doubled over the years since 1988, and 50% of the 2013 meetings had at least one female organizer. Banbury meetings continue to have strong international participation with 18% of participants coming from 20 countries.

One of the postponed meetings was on *Redesigning Photosynthesis—Identifying Opportunities and Novel Ideas,* organized by Donald Ort (University of Illinois) and Sabeeha Merchant (University of California, Los Angeles). Nearly all other biological processes on earth depend on the ability of photosynthesis to convert solar energy into chemical energy. There is a great deal of interest in the efficiency with which photosynthesis can accomplish this as it is the basis of the yield potential of both our food and bioenergy crops. In fact, photosynthesis is rather inefficient when all the costs are factored in; in the world's best agricultural regions, ~1% of the total solar energy that falls on the field during the growing season is stored as chemical energy in the plant materials at the end of the season. Participants discussed whether the efficiency of solar energy capture by photosynthesis could be improved even though evolution has provided very little genetic variation in the component mechanisms of photosynthesis.

Conference Room, Fall 2013

The second plant science meeting in 2013 also dealt with metabolism. Organized by Toni Kutchan (Danforth Center, St. Louis), Robert Last (Michigan State University), and Anne Osborn (John Innes Centre, United Kingdom), *Evolution of Plant Metabolic Diversity* focused on the evolution of specialized metabolism in plants. Most classes of specialized compounds are taxonomically restricted, making their analysis less accessible to some of the traditional tools of biology. However, studies of diverse plants, including "nonmodel" species, are benefiting greatly from recent advances in genomics, metabolomics, reverse genetics, and synthetic biology. These tools are allowing rapid enzyme discovery and pathway identification, and the abundance of data across and within taxa creates unprecedented opportunities for comparative analysis. The meeting brought together leaders in studies of these biosynthetic pathways and their functions, along with researchers at the forefront of comparative genomics, evolution, systems, and synthetic biology.

The Banbury Center is known for having meetings on what might be called "emerging topics," and four such meetings took place in 2013. The first was organized by Joshua Dubnau (Cold Spring Harbor Laboratory) and Fred Gage (Salk Institute for Biological Studies). Transposable elements are mobile genetic elements that constitute approximately 50% of the human genome. Some transposable elements have been shown to cause neurodegenerative diseases by insertional mutagenesis, but very recently there have been reports that transposable elements are active during *normal* neurogenesis. This suggests that mobilization of transposable elements in the developing brain might contribute to neuronal diversification. If transposable element mobilization is important in normal brain development, we may need to revise the way we think about the brain.

The involvement of telomeres in aging and aging-related disorders has been known since Carol Greider and Bruce Futcher here at CSHL, together with Calvin Harley at McMaster University, showed that telomeres shorten as human diploid fibroblasts age in cell culture. More recently, a growing body of evidence is implicating telomeres in the pathogenesis of several important degenerative disorders including pulmonary fibrosis, bone marrow failure, and diabetes. However, the underlying role of telomeres in these diverse disorders is not well understood. Is the role of shortened telomere length in these disorders due to effects on stem cells? What is the relationship between telomeres, mitochondria, and cell death? Can measurement of telomere length be a useful diagnostic tool? Will an understanding of the role of telomeres in these disorders point to new therapeutic strategies? Organized by Mary Armanios (Johns Hopkins

Meier House, Winter 2013

Sammis, Winter 2013

University) and Peter Lansdorp (University of Groningen), the meeting brought together scientists and clinicians to review and critically assess current data on how telomere dysfunction contributes to these diseases.

Enhancers—transcriptional regulatory elements that, as their name suggests, enhance gene expression—have been studied for many years. In recent years, there has been rapid progress in identifying transcriptional regulatory elements and the factors that occupy them. In particular, "superenhancers," large clusters of transcriptional enhancers, have been identified. Disease-associated sequence variation occurs in some of these regulatory elements and in the factors that bind them. *Enhancer Biology in Health and Disease*, organized by James Bradner (Dana Farber Cancer Institute), Joanna Wysocka (Stanford University), and Richard Young (Whitehead Institute), brought together experts in enhancer biology to discuss the roles of superenhancers in controlling gene expression and their impact on human health and disease. Topics included basic biology of enhancers (enhancers and chromatin folding, and enhancer dynamics), reviews of the evidence for enhancer involvement in diseases, and how to perform large-scale functional analysis of enhancers identified by sequencing.

Finally, it was a pleasure to have a former Watson School of Biological Sciences student become an organizer of a Banbury Center meeting. Yaniv Erlich left the WSBS with a PhD in 2010 and is now at the Whitehead Institute for Biomedical Research in Cambridge, Massachusetts. Yaniv and his colleagues caused something of a sensation in early 2013, when they published a paper showing that it was possible to recover surnames associated with supposedly anonymized sequence data, using public, freely accessible Internet resources. One concern is whether the ability to do this, even on a limited scale, might lead to restrictions on the availability of genome data. The goals of *Accelerate Genomic Research with Privacy Protections*, organized by Yaniv, Arvind Narayanan (Princeton University), and Robert Kain (Illumina Inc.), were to discuss technical strategies for maintaining privacy of genetic and –omics data sets so that future research would not be compromised. Participants were drawn from an especially wide range of disciplines—human genetics, bioinformatics, cryptography, and ethics.

The continuing success of the Banbury Center program is due to the efforts of many people. Janice Tozzo and Pat Iannotti in the Banbury office, Basia Polakowski at Robertson House, and Jose Pena Corvera, Fredy Vasquez, and Joe McCoy looking after the grounds, all worked

very hard to keep the Center running smoothly. Culinary Services, Facilities, and the Meetings Office played key roles in the operation of the Center. The meetings could not take place without the hard work of the organizers, the generosity of the Laboratory's Corporate Sponsors and the other donors who funded our meetings, and the Laboratory's scientists who continue to support the Center.

Jan Witkowski
Executive Director

Cocktails at the Robertson House

BANBURY CENTER MEETINGS

	Title	*Organizer(s)*
February 3–6	Oxidants and Antioxidants in Cancer Genesis and Treatment	Toren Finkel, Nicholas Tonks, David Tuveson
February 19–22	Interdisciplinary Approaches to Idiopathic Lung Fibrosis	Brigid Hogan, Maria Padilla
February 28–March 3	Grand Challenges in Organismal Biology: Walking the Tightrope between Stability and Change	Dianna Padilla, Billie Swalla, Brian Tsukimura
March 3–6	Evolution of Plant Metabolic Diversity	Toni Kutchan, Robert Last, Anne Osbourn
March 31–April 2	Transposable Elements in the Brain and Other Tissues: Prevalence and Function	Joshua Dubnau, Fred Gage
April 14–16	Development and Evolution of the Human Motor System in Relation to ALS and FTD	Lucie Bruijn, Jeffrey Macklis, Martin Turner
April 19–24	Communicating Science	Sandra Schedler, Claudia Walther
April 28–30	Developing a Neuroscience Consortium	Larry Alphs, Arthur Holden
May 13–16	Redesigning Photosynthesis: Identifying Opportunities and Novel Ideas	Sabeeha Merchant, Donald Ort
July 14–16	The Emerging Intersection between Physical Sciences and Oncology	David Agus, Danny Hillis, Parag Mallick,
September 8–11	Telomeres and Disease	Mary Armanios, Peter Lansdorp
September 15–18	Neurobiology and Clinical Study of Rapid-Acting Antidepressants	Ronald Duman, Carlos Zarate
September 22–25	Plant Reproduction	Robert Martienssen, Robert Meeley
September 29–October 1	Science of Pancreatic Cancer	Ronald Evans, William Isacoff, David Tuveson
October 6–9	Biguanides and Neoplasia	Michael Pollak, Kevin Struhl
October 21–22	Lustgarten Foundation Scientific Meeting	Mila McCurrach
October 23–25	Ovarian Cancer: Developing Research-Based Public Messaging on Early Detection and Screening	Jeffrey Boyd, Audra Moran, Michael Seiden
October 27–30	Enhancer Biology in Health and Disease	James Bradner, Joanna Wysocka, Richard Young
November 12–15	INK4a/ARF Network	David Beach, Norman Sharpless. Charles J. Sherr
December 3–5	The Adolescent Brain	Jay Giedd, Hakon Heimer, Edward Lein, Nenad Sestan
December 8–11	Psychiatric Genomics: Current Status, Future Strategies	W. Richard McCombie, Aarno Palotie
December 11–13	Accelerate Genomic Research with Privacy Protections	Yaniv Erlich, Robert Kain, Arvind Narayanan
December 15–17	Phelan-McDermid Syndrome: Autism Due to Shank3 Mutations/Deletions	Geraldine Bliss, Ricardo Dolmetsch, Craig Powell

BANBURY CENTER MEETINGS

Oxidants and Antioxidants in Cancer Genesis and Treatment

February 3–6

FUNDED BY **John K. Castle**, **Oliver Grace Cancer Fund**

ARRANGED BY **T. Finkel,** National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland
N. Tonks, Cold Spring Harbor Laboratory
D. Tuveson, Cold Spring Harbor Laboratory

Increased production of reactive oxygen species (ROS) has been functionally linked to aging and cancer. It is now clear that the cellular antioxidant machinery can be up-regulated in response to oncogenes and may confer drug resistance and "stemness." Such observations suggest that redox modulation may offer a new approach for selective targeting of cancer cells. Participants in this meeting explored the chemical, biochemical, and genetic facets of ROS biology in relation to cancer, with the goal of determining whether ROS can be manipulated in vivo to alter cancer pathogenesis and the response of cancer cells to therapy.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Pathways and Redox

Chairperson: T. Finkel, National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland

T. Finkel, National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland: Oxidants as signaling molecules.

B. Burgering, University Medical Center Utrecht, The Netherlands: Redox control of FOXO transcription factors to balance life span with disease.

B. Wouters, Ontario Cancer Institute, Toronto, Canada: The role of unfolded protein response on autophagy and ROS during hypoxia.

SESSION 2: Redox Biology

Chairperson: P. Schumacker, Northwestern University, Chicago, Illinois

P. Huang, MD Anderson Cancer Center, Houston, Texas: ROS stress in cancer: Mechanisms and therapeutic implications.

M. Espey, National Cancer Institute/NIH, Bethesda, Maryland: Interplay between mechanobiology, NO, and O_2 in tumors.

SESSION 3: ROS Biology and Chemistry

Chairperson: M. Murphy, University of Cambridge, United Kingdom

M. Murphy, MRC Mitochondrial Biology Unit, Cambridge, United Kingdom: Exploring mitochondrial ROS with targeted molecules.

P. Schumacker, Northwestern University, Chicago, Illinois: Mitochondrial oxidant signals trigger stress responses

N. Chandel, Northwestern University, Chicago, Illinois: Mitochondria regulate cancer.

SESSION 4: ROS Methods and Therapeutic Development

Chairperson: J. Held, Buck Institute for Age Research, Novato, California

J. Held, Buck Institute for Age Research, Novato, California: Mass spectrometric approaches to characterize oxidized cysteines.

T. Dick, German Cancer Research Center, Heidelberg, Germany: In vivo ROS imaging: Concepts, limitations, future directions.

M. Adorno, Institute for Stem Cell Biology & Regenerative Medicine, Stanford University, California: Trisomy of Usp16 contributes to senescence and stem cell defects in somatic tissues of Down syndrome and is associated with breast cancer protection.

Summary Discussion, T. Finkel, National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland

SESSION 5: Exogenous and Endogenous Antioxidants and Cancer

Chairperson: A. Holmgren, Karolinska Institute, Stockholm, Sweden

A. Holmgren, Karolinska Institute, Stockholm, Sweden: Thioredoxin and glutaredoxin systems in DNA synthesis and control of cell death.

T. Mak, Princess Margaret Hospital, Toronto, Canada: Regulation of oxidants and antioxidants in oncogenic metabolic adaption.

G. Buettner, University of Iowa, Iowa City: Applying quantitative redox biology to understand mechanisms of pharmacological ascorbate in cancer treatment.

SESSION 6: NRF2 and ROS Regulation

Chairperson: J. Hayes, Ninewells Hospital and Medical School, Dundee, United Kingdom

J. Hayes, Ninewells Hospital and Medical School, Dundee, United Kingdom: Roles of transcription factor Nrf2 in adaptation to oxidative stress and tumor growth.

S. Biswal, Johns Hopkins School of Public Health, Baltimore, Maryland: Nrf2 at the crossroad of redox and energy metabolism and therapeutic resistance.

M. Sporn, Dartmouth Medical School, Hanover, New Hampshire: Nrf2: Good or bad for cancer?

SESSION 7: ROS Signaling

Chairperson: S.G. Rhee, Ewha Womans University, Seoul, Korea

S.G. Rhee, Ewha Womans University, Seoul, Korea: Centrosomal accumulation of H_2O_2 through Peroxiredoxin I inactivation is required for mitotic entry.

A. Ostman, Karolinska Institute, Stockholm, Sweden: ROS-mediated regulation of cell signaling through oxidation of tyrosine phosphatases.

S. Muthuswamy, Ontario Cancer Institute, University of Toronto, Canada: Cell polarity, protein scribble, and ROS.

SESSION 8: ROS and p53

Chairperson: K. Vousden, Beatson Institute, Glasgow, United Kingdom

K. Vousden, Beatson Institute, Glasgow, United Kingdom: The role of p53 in regulation of ROS.

R. Sordella, Cold Spring Harbor Laboratory: p53 mutation or splice forms cause mitochondrial ROS.

Summary Discussion: A. Holmgren, Karolinska Institute, Stockholm, Sweden

SESSION 9: ROS and Therapies I

Chairperson: A. Letai, Dana-Farber Cancer Institute, Boston, Massachusetts

I. Blair, University of Pennsylvania, Philadelphia: Serum biomarkers of oxidative stress.

A. Letai, Dana-Farber Cancer Institute, Boston, Massachusetts: Mitochondrial fitness and response to anticancer therapy.

R. Sordella, J. Schlessinger

K. Vousden

G. Wondrak, University of Arizona, Tucson: Teaching old dogs new tricks: Drug repurposing for redox-directed cancer chemotherapy.

D. Spitz, University of Iowa, Iowa City: Metabolic oxidative stress in cancer biology and therapy.

SESSION 10: ROS and Therapies II

Chairperson: B. Stockwell, Columbia University, New York

B. Stockwell, Columbia University, New York: Ferroptosis: An iron-dependent, oxidative form of nonapoptotic cell death.

Final Meeting Summary: D. Tuveson and N. Tonks, Cold Spring Harbor Laboratory

D. Tuveson, N. Chandel

Interdisciplinary Approaches to Idiopathic Lung Fibrosis

February 19–22

FUNDED BY **Elizabeth Livingston Estate**

ARRANGED BY **B. Hogan,** Duke University Medical Center, Durham, North Carolina
M. Padilla, Mount Sinai School of Medicine, New York

M. Padilla, B. Hogan

Idiopathic pulmonary fibrosis (IPF) is a devastating clinical condition for which there is no effective therapy. This meeting brought together an eclectic mix of clinical and basic scientists with the goal of stimulating ideas about the origin and progression of fibrotic lesions in IPF and how new research tools and experimental paradigms can be developed to test them and to move basic studies into the clinic. Sessions followed the traditional Banbury format of short talks and discussion and focused extended discussions. Topics covered included clinical progression and heterogeneity of IPF; genetic and genomic approaches; development of the peripheral lung, including lineage tracing and new mouse models; stem cells and their regeneration in relation to fibrosis; new discoveries related to fibrosis in different organ systems; role of oxidative and ER stress and senescence; the molecular biology of myofibroblasts, pericytes, and other mesenchymal cells; complexities of the extracellular matrix and signaling pathways; the role of immune cells; and progress of clinical trials.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: B. Hogan, Duke University Medical Center, Durham, North Carolina

SESSION 1: Overviews of Clinical Impact, Pathology, and Classification of Idiopathic Pulmonary Fibrosis

M. Padilla, Mount Sinai School of Medicine, New York: Overview of idiopathic pulmonary fibrosis and other fibrosing interstitial pneumonias: Clinical challenges.

K. Leslie, Mayo Clinic, Scottsdale, Arizona: The pathology of idiopathic pulmonary fibrosis, what have we learned in 50 years?

P. Noble, Duke University School of Medicine, Durham, North Carolina: Overview of pathologic heterogeneity in idiopathic pulmonary fibrosis.

Issues to Consider for Meeting

SESSION 2: Genetics and Genomic Approaches to Lung Fibrosis

C. K. Garcia, University of Texas Southwestern Medical Center, Dallas: Genetic heterogeneity of idiopathic pulmonary fibrosis.

S. Guttentag, University of Pennsylvania, Philadelphia: Pulmonary fibrosis in Hermansky Pudlak syndrome

N. Kaminski, University of Pittsburgh Medical Center, Pennsylvania: Novel biomarkers for idiopathic pulmonary fibrosis.

General Discussion

SESSION 3: Cellular Stress, Senescence, and Epithelia-Mesenchymal Interactions in Relation to Fibrosis

M. Armanios, Johns Hopkins University, Baltimore, Maryland: Telomerase and idiopathic pulmonary fibrosis.

S. Savage, National Cancer Institute, Bethesda, Maryland: Dyskeratosis congenita as a model for understanding pulmonary fibrosis.

S. Friedman, Mount Sinai Hospital, New York: Autophagy drives fibrogenic cell activation in tissue injury?

SESSION 4: Fibrosis in Multiple Organ Systems

J. Duffield, University of Washington, Seattle: Kidney, pericytes, and lung fibrosis.

L. Sakai, Shriners Hospital for Children, Portland, Oregon: Fibrosis due to mutations in fibrillin-1.

SESSION 5: Embryonic Development of the Alveolar Regions of the Lung, Lung Stem Cells, and Alveolar Cell Interactions

E. Morrisey, University of Pennsylvania, Philadelphia: Contribution of Wnt2+ mesoderm progenitors to the developing and adult lung.

B. Hogan, Duke University Medical Center, Durham, North Carolina: Alveolar stem cells and fibrosis in the mouse lung.

B. Hinz, University of Toronto, Canada: Myofibroblast mechanics.

R. Chambers, Centre for Respiratory Research, Rayne Institute, London, England: Coagulation cascade and pulmonary fibrosis.

SESSION 6: Proliferation and Differentiation of Myofibroblasts and Other Mesenchymal Cells (Pericytes, Vascular Smooth Muscle, Lipofibroblasts, Fibrocytes) and Matrix

P. Noble, Duke University School of Medicine, Durham, North Carolina: Regulation of severe pulmonary fibrosis: Roles of the ECM.

L. Olson, Oklahoma Medical Research Foundation, Oklahoma City: PDGF signaling and fibrosis.

K. Leslie, P. Ward, B. Stillman

SESSION 7: Immune System and Lymphatics

I. Rosas, Brigham and Women's Hospital, Boston, Massachusetts: NLRP3 inflammasome activation and experimental pulmonary fibrosis.

C. Becker, Mount Sinai School of Medicine, New York: The role of dendritic cells in idiopathic pulmonary fibrosis.

W. Bradford, InterMune, Inc., Brisbane, California: Idiopathic pulmonary fibrosis clinical trial efficiency.

P.A. Ward, University of Michigan, Ann Arbor: Regulation of the lung inflammatory response by the adrenergic and cholinergic nervous systems.

C. Hogaboam, University of Michigan, Ann Arbor: Innate immune signaling and fibrosis.

D. Wilkes, Indiana University School of Medicine, Indianapolis: Autoimmunity to type V collagen in idiopathic pulmonary fibrosis pathogenesis.

General Discussion

SESSION 8: Clinical Trials

S. Violette, Biogen Idec, Cambridge, Massachusetts: Developing a biomarker strategy to support the early clinical development of STX-100 in IPF patients.

S. Friedman, Mount Sinai Hospital, New York: Defining therapeutic targets for antifibrotic therapies: Challenges and opportunities.

General Discussion and Wrap Up, Potential Areas for Future Collaboration

Impromptu seminar

Grand Challenges in Organismal Biology: Walking the Tightrope between Stability and Change

February 28–March 3

FUNDED BY **Stony Brook University through a grant from the National Science Foundation**

ARRANGED BY **D. Padilla,** Stony Brook University, New York
B. Swalla, University of Washington, Seattle
B. Tsukimura, California State University, Fresno

A central paradox in biology is that animals must maintain the integration of complex developmental and functional systems while simultaneously responding and adapting to continuously changing internal and external environments. Understanding how animals maintain the balance between integrated stability and flexibility (both short-term accommodation and long-term evolutionary adaptation) is of growing importance. However, we do not understand the functional and system-level attributes of animals that make them resilient or robust to internal or external environmental perturbation or, conversely, sensitive or fragile. In particular, we need to understand mechanisms that mediate phenotypic responses to environmental inputs across different scales and to develop quantitative frameworks for analyzing these phenomena. Participants identified critical areas and questions that require new information or approaches, and priorities for new research agendas to address this grand challenge.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

I. **Introduction of the Grand Challenge of Organismal Biology**
   a. History from NSF to this meeting
      i. GCOB in general
      ii. Selection of walking a tightrope as a first effort
   b. Discussion of Steering Meeting: Goals and challenges
   c. Vision Statement: Transformation of community
   d. Guidelines and deliverables from this big meeting

II. **Question and Answer Session**
III. **What Have Other Communities Done That Are Successful?**
   a. NCEAS example: Building the next generation of scientist
   b. iPlant
IV. **Tutorial: Control Theory (T. Daniel and N. Cowan)**
   a. A control theorist's view on organismal biology (stability): A tutorial
   b. How can control theory be used to answer this GCOB?
   c. How do dynamics interact among scales?
V. **Instructions and Ground Rules for Breakout Sessions**
VI. **First Breakout Session**
   What are the most important, burning questions explicitly across temporal, spatial, and organizational scales related to stability and change?
   What new approaches can/should we use to address these questions?
VII. **Second Breakout Session**
   What are the major challenges or impediments to addressing the burning questions beyond capacity building or just more funding in general?
   Are there specific needs or approaches that would jump-start making progress (e.g., specific national opportunities to facilitate and amplify interactions, targeted research centers)? What they would target, RCNs for mining existing data, and what areas would be most profitable?
   What would your wish list be for solving these questions?
VIII. **Third Breakout Session**
   What are the mutual benefits and deliverables of a new set of approaches for organismal biology, recognizing that the dynamics occurring on all levels of biological organization are inextricably linked?
IX. **Fourth Breakout Session**
   Given steering committee recommendations, what are the short-term and long-term best ways to move forward to answering this GCOB?
   What are the most easily accomplished (low-hanging fruit) and targeted funding priorities?
X. **Grand Synthesis of What We Have Accomplished**
XI. **Next Steps**

D. Plachetzki, D. Grunbaum, Z. Cheviron, J. Marden, M. Hale

Evolution of Plant Metabolic Diversity

March 3–6

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **T. Kutchan,** Danforth Center, St. Louis, Missouri
R. Last, Michigan State University, East Lansing
A. Osbourn, John Innes Centre, Norwich, United Kingdom

This is an exciting time for investigation of specialized (secondary) metabolism in plants. Most of these specialized compounds are taxonomically restricted, making their analysis less accessible to some of the traditional tools of biology. However, studies of diverse plants are benefiting greatly from recent advances in genomics, metabolomics, reverse genetics, and synthetic biology. The abundance of data across and within taxa is creating unprecedented opportunities for comparative analysis. Given the important ecological functions of these molecules, it is not surprising that examples of evolutionary plasticity and strong phenotypic diversity are being uncovered for a variety of biosynthetic pathways. This meeting focused on the evolution of specialized metabolism in plants. Participants included leaders in studies of biosynthetic pathways and researchers at the forefront of comparative genomics, evolution, systems, and synthetic biology.

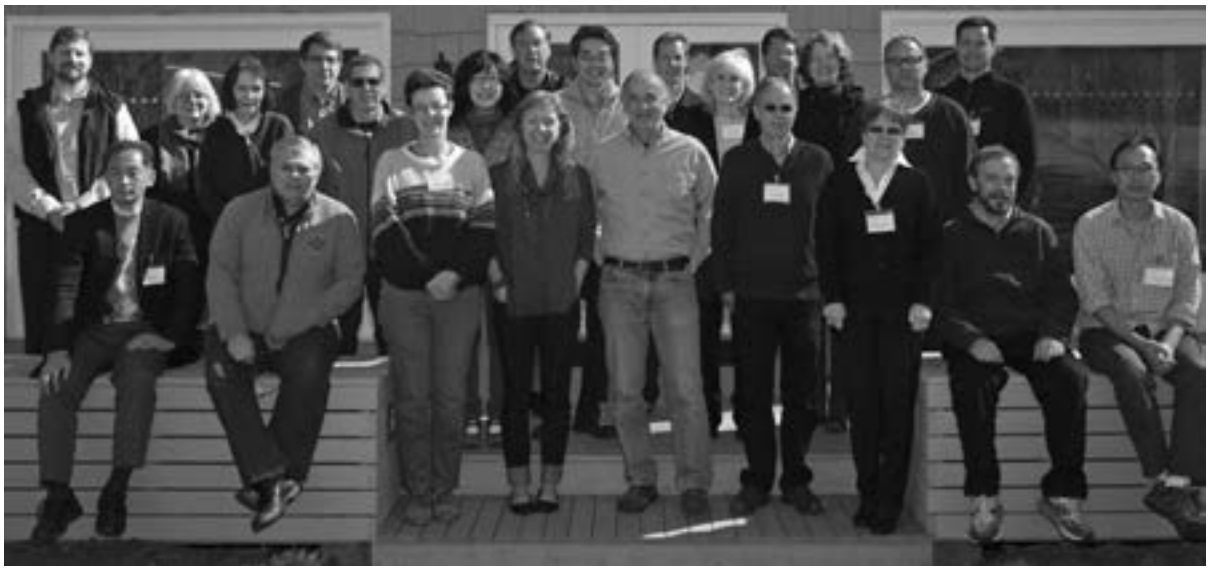
Welcoming Remarks: **J.A. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

Introductory Remarks: **R. Last,** Michigan State University, East Lansing

SESSION 1: Metabolite Diversity in an Evolutionary/Functional Context

Chairperson: T. Kutchan, Danforth Center, St. Louis, Missouri

R. Last, Michigan State University, East Lansing: Signatures of evolution in glandular trichomes of *Solanum*.



K. Saito, Riken Plant Science Center, Chiba University, Japan: Origin of metabolomic diversity.
M. Simmonds, Royal Botanic Gardens, Kew, London, England: Plant chemosystematics: New opportunities for selecting plants.
E. Kellogg, University of Missouri, St. Louis: Secondary metabolism in the Poaceae (grasses).
T. Mitchell-Olds, Duke University, Durham, North Carolina: Metabolism and complex traits.

General Discussion

SESSION 2: Regulation of Metabolism

Chairperson: A. Osbourn, John Innes Centre, Norwich, United Kingdom

N. Doudareva, Purdue University, West Lafayette, Indiana: An alternate microbial pathway contributes to phenylalanine biosynthesis in plants.
D.J. Kliebenstein, University of California, Davis: Evolution of regulatory links between primary and secondary metabolism.
H. Klee, University of Florida, Gainesville: Regulation of flavor-associated chemical accumulation in the tomato fruit.
M. Lange, Washington State University, Pullman: Evolution of specialized plant tissues and cell types for the synthesis and accumulation of terpenoids.
V. DeLuca, Brock University, Ontario, Canada: Specialized metabolism and the recruitment of multiple cell types for functional pathway organization.

General Discussion

SESSION 3: Pathway Evolution

Chairperson: R. Last, Michigan State University, East Lansing

A. Osbourn, John Innes Centre, Norwich, United Kingdom: Pathway evolution.
E. Pichersky, University of Michigan, Ann Arbor: Terpene gene evolution, evolution of functional gene clusters.
D. Werck-Reichhart, Institute of Plant Molecular Biology, Strasbourg, France: Cytochromes P450 as landmarks of plant metabolism evolution.
E. Wurtzel, City University of New York–Lehman College, Bronx, New York: Enzyme evolution and topological control of carotenoid biosynthesis in plants.
E. Cahoon, University of Nebraska, Lincoln: Evolution of unusual fatty acid synthesis: The case of acetylenic fatty acids and polyacetylenes.

General Discussion

SESSION 4: Omics Approaches to Studies of Pathway and Genome Evolution

Chairperson: H. Klee, University of Florida, Gainesville

S.-H. Shiu, Michigan State University, East Lansing: Metabolic gene duplication and functional divergence/convergence.
S. Rhee, Carnegie Institution of Washington, Stanford, California: Genomic signatures of specialized metabolism evolution in plants.
T. Mockler, Donald Danforth Plant Science Center, St. Louis, Missouri: Informing metabolic studies using transcriptome profiling.
T. Kutchan, Danforth Plant Science Center, St. Louis, Missouri: Using genomics to elucidate biochemical pathways.

General Discussion

SESSION 5: Applying Evolutionary Principles to Pathway Engineering

T. Mitchell-Olds

C. Paddon, E. Wurtzel

Chairperson: K. Saito, Riken Plant Science Center, Chiba University, Japan

I. Abe, University of Tokyo, Japan: Engineered biosynthesis of plant polyphenols.

C. Paddon, Amyris, Inc., Emeryville, California: Semisynthetic Artemisinin: Using synthetic biology to increase the supply of a crucial antimalarial drug.

R. Peters, Iowa State University, Ames: To gibberellins and beyond! The evolution of (Di)terpenoid metabolism.

J. Noel, Salk Institute for Biological Studies, La Jolla, California: The remarkable pliability and promiscuity of specialized metabolism.

Summary Discussion

Conference Room, Winter 2013

Transposable Elements in the Brain and Other Tissues: Prevalence and Function

March 31–April 2

FUNDED BY **Dart NeuroScience and the Marie Robertson Research Fund**

ARRANGED BY **J. Dubnau,** Cold Spring Harbor Laboratory
F. Gage, Salk Institute for Biological Studies, San Diego, California

The functions, if any, of transposable elements (TEs) in the human genome are largely unknown. However, they do cause disease by insertional mutagenesis and have been linked to neurodegenerative diseases. These include transmissible prion disorders, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, macular degeneration, fragile-X-tremor ataxia, and normal aging. There are recent reports that several types of TEs are active during *normal* neurogenesis in mammals and invertebrates. It has been suggested that active mobilization of transposable elements in the developing brain can produce somatic neuronal genetic heterogeneity and that this somatic variation may contribute to neuronal diversification. Participants critically reviewed the state of the field, and the meeting concluded with a session devoted to considering future lines of research, discussing how to promote this area of research, and how to encourage funding by foundations and NIH.

F. Gage, H. Kazazian, A. Ferguson-Smith

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: J. Dubnau, Cold Spring Harbor Laboratory

SESSION 1: Transposons and Genome Evolution

Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

R. Martienssen, Cold Spring Harbor Laboratory: Transposons at Cold Spring Harbor Laboratory

P. Batut, Cold Spring Harbor Laboratory: Transposons and evolution of transcriptional regulation in the *Drosophila* clade.

A. Ferguson-Smith, University of Cambridge, United Kingdom: Large clusters of LINE1 repeats: Functional or junk?

K. Kosik, University of California, Santa Barbara: The emergence of brain noncoding RNAs at the Catarrhini Branch.

K. Burns, Johns Hopkins University School of Medicine, Baltimore, Maryland: Mapping and functional analysis of transposable element insertions.

M.C. Marchetto, Salk Institute for Biological Studies, La Jolla, California: Differential LINE-1 retrotransposition in induced pluripotent stem cells between humans and great apes.

SESSION 2: Functional Roles and Regulatory Mechanisms

Chairperson: F. Gage, Salk Institute for Biological Studies, San Diego, California

O. Voinnet, Swiss Federal Institute of Technology Zurich, Switzerland: RNAi-dependent and -independent control of LINE1 mobility and accumulation in mouse ES cells.

K. Creasey, Cold Spring Harbor Laboratory: Plants catch transposons in the act: Control when methylation fails.

S. Waddell, University of Oxford, England: Transposition-driven genomic heterogeneity in the *Drosophila* brain.

C. Walsh, Boston Children's Hospital, Cambridge, Massachusetts: Single-neuron, whole-genome analysis of L1 retrotransposition in the human brain.

A. Muotri, University of California, San Diego, La Jolla: Impact of L1 retrotransposition in the nervous system.

J. Moran, University of Michigan Medical School, Ann Arbor: Studies of a human retrotransposon.

P. Batut photographing Barbara McClintock's corn cobs

SESSION 3: Dysfunction and Disease

Chairperson: S. Martin, University of Colorado, Aurora

J. Dubnau, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York: Jumping into neurodegeneration.

R. Hunter, Rockefeller University, New York: Stress and hippocampal transposable element expression.

P. Jin, Emory University School of Medicine, Atlanta, Georgia: Transposable elements in neurodegeneration.

H. Kazazian, Johns Hopkins University, Institute of Genetic Medicine, Baltimore, Maryland: Extensive somatic L1 retrotransposition in colon cancer.

A. Nath, National Institutes of Health, Bethesda, Maryland: Human endogenous retroviruses in ALS.

Summary, Discussion, and Future Research

Development and Evolution of the Human Motor System in Relation to ALS and FTD

April 14–16

FUNDED BY **Greater New York Chapter of the ALS Association**

ARRANGED BY **L. Bruijn,** ALS Association, Washington, DC
J. Macklis, Harvard University, Cambridge, Massachusetts
M. Turner, University of Oxford, United Kingdom

The neurodegenerative process characteristic of amyotrophic lateral sclerosis (ALS) may be regarded as a system failure on several levels. The mechanisms of spread and of the variable penetrance of pathology within extramotor, upper and lower motor neuronal populations (and supporting cells) remain uncertain, but they are critical to hopes of therapeutic intervention. Data suggest that wider cortical organization, local circuits, and developmental factors may be important in defining vulnerability to neurodegenerative disorders. The neocortical evolutionary changes involved in bipedalism with opposable thumbs, and the relative athleticism observed premorbidly among patients, have been postulated to hold particular relevance for ALS. An understanding of the development and evolution of the motor system and its frontotemporal connections has the potential to re-frame thinking on the pathogenesis of both ALS and FTD. This symposium was the first to draw together a multidisciplinary group of internationally leading neuroscientists who might not otherwise interact.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: L. Bruijn, ALS Association, Washington, DC

SESSION I

Chairperson: J. Macklis, Harvard University, Cambridge, Massachusetts

M. Turner, University of Oxford, John Radcliffe Hospital, United Kingdom: Introduction: From Charcot to C90rf72.

K. Talbot, University of Oxford, John Radcliffe Hospital, United Kingdom: The clinical spectrum of disorders affecting the motor neuron.

V.R. Edgerton, University of California, Los Angeles: Evolutionary organization of motor control circuitry.

A. Eisen, University of British Columbia, Canada: Evolutionary considerations in the pathogenesis of ALS: A clinical perspective.

SESSION II

Chairperson: J. Rothstein, Johns Hopkins University School of Medicine, Baltimore, Maryland

J. Martin, City College of New York, New York: Circuit function: Corticospinal and descending systems.

J. Macklis, Harvard University, Cambridge, Massachusetts: Molecular logic of corticospinal motor neuron development and broader neuron class evolution.

W. Seeley, University of California, San Francisco: Selective neuronal and network-based vulnerability in frontotemporal dementia.

SESSION III

Chairperson: J. Ravits, University of California, San Diego

Z. Molnár, University of Oxford, United Kingdom: The earliest cortical circuits.

S. Pfaff, Salk Institute for Biological Studies, La Jolla, California: Spinal motor neuron development.

J. Rothstein, Johns Hopkins University School of Medicine, Baltimore, Maryland: Cell vulnerability and the role of non-neuronal cells and interneurons in MND.

G. Miles, University of St. Andrews, Fife, Scotland, United Kingdom: Physiology and pathology of spinal motor circuitry.

R. Brownstone, Dalhousie University, Halifax, Nova Scotia, Canada: Control of spinomuscular circuits.

M. Hallett, National Institute of Neurological Disorders, Bethesda, Maryland: Spinal cord circuitry and function.

SESSION IV

Chairperson: A. Al Chalabi, Kings College, Institute of Psychiatry, London, England

A. Al Chalabi, Kings College, Institute of Psychiatry, London, England: Introductory remarks to tie the session together.

D.W. Dickson, Mayo Clinic, Jacksonville, Florida: The emerging neuropathological taxonomy of ALS and FTD. The range of ALS and FTD phenotypes and their overlap: A pathological view.

M. Strong, Schulich School of Medicine & Dentistry, Ontario, Canada: The emerging neuropsychological spectrum of frontotemporal dysfunction in ALS.

SESSION V

Chairperson: M. Benatar, University of Miami Hospital, Miller School of Medicine, Florida

J. Ravits, University of California, San Diego: Clinicopathological observations on spread in ALS.

A. Eisen

S. Pfaff, J. Rothstein

J. Shefner, State University of New York, Upstate Medical University, Syracuse: Lower motor neuron studies evaluating spread of disease burden.
G. Fishell, New York University Medical Center, New York: Interneuron development.
E. Azim, Columbia University, New York: Genetic manipulation of circuits for skilled forelimb movement in mice.
M. Kiernan, Institute of Neurological Sciences, Neuroscience Research of Australia, Sydney: Cortical excitability in ALS.
T. Siddique, Northwestern University, Feinberg School of Medicine, Chicago, Illinois: Is neurodegeneration a consequence of protein in evolutionary conflict?

General Discussion

Closing Remarks

SESSION VI

Chairperson: T. Maniatis, Columbia University Medical Center, New York

Conference Room

Communicating Science

April 19–24

FUNDED BY **Boehringer Ingelheim Fonds Foundation for Basic Research in Medicine**

ARRANGED BY **S. Schedler,** Boehringer Ingelheim Fonds, Mainz, Germany
C. Walther, Boehringer Ingelheim Fonds, Mainz, Germany

The Boehringer Ingelheim Fonds (BIF) has an international program of support for PhD fellowship, and 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 2013 stay at Banbury was the sixth occasion on which they have been here. At Banbury, the fellows 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 and All About BIF
C. Walther, Boehringer Ingelheim Fonds, Mainz, Germany

Communication in General
N. LeBrasseur, DNA Medical Communications, New York, New York
Writing techniques and how to structure papers

Preparing and Delivering a Scientific Talk
B. Tansey, Vanderbilt University Medical Center, Nashville, Tennessee

How to Measure Success in Science
R. Lehmann, New York University School of Medicine, New York, New York

How to Design Figures
K. Ris-Vicari, Katie Ris-Vicari Graphic Design, Levittown, New York and **Matt Hansen**, Nature Publishing Group, New York, New York

Developing a Neuroscience Consortium

April 28–30

FUNDED BY **ISCTM and Individual Participants**

ARRANGED BY **L. Alphs,** Janssen Pharmaceuticals, Titusville, New Jersey
A. Holden, Pharmaceutical Biomedical Research, Chicago, Illinois

There has been considerable support for developing a consortium to combine existing industry databases (with the goal of including quality data from other sources as well). Given the level of interest and support, this meeting was convened to discuss the practical issues involved and what can be done to move the project forward. The topics that were reviewed included the goals for the Neuroscience Consortium; what might be the organizational structure of the Consortium and how might it relate to or even be integrated into existing organizations; the legal and intellectual hurdles that must be overcome to make this organization successful; the possible financial models for this organization; and the major milestones and timeline for the successful development of this consortium.

L. Alphs, M. Burke

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction and Outline of the Meeting: L. Alphs, Janssen Pharmaceuticals, Titusville, New Jersey and
A. Holden, Pharmaceutical Biomedical Research, Chicago, Illinois

SESSION 1: Rationale for Consortium: The Value of Big Neuroscience Clinical Data

Goal: Brainstorming on What the Precompetitive Neuroscience Data-Sharing Consortium Might Do

Brief presentations followed by brief discussions that summarize a variety of potential different uses of consortium data.

W. Potter, National Institute of Mental Health, Bethesda, Maryland: The value of big neuroscience clinical data: A perspective from NIH.

Discussion

J. Dudley, Mount Sinai School of Medicine, New York: The value of big neuroscience clinical data: A computational biologist's perspective.

Discussion

A. Cross, AstraZeneca Pharmaceuticals, Cambridge, Massachusetts: The value of big neuroscience clinical data: A drug developer's perspective.

Discussion

H. Geerts, Silico Biosciences, Philadelphia, Pennsylvania: The value of big neuroscience clinical data: A disease modeler's perspective.

Discussion

M. Arrighi, Janssen Research and Development, South San Francisco, California: (presented by Larry Alphs)

The value of big neuroscience clinical data: An epidemiologist's perspective.

Discussion

D. Meltzer, University of Chicago, Illinois: The value of big neuroscience clinical data: An economist's perspective.

Discussion

W.R. McCombie, Cold Spring Harbor Laboratory: The value of big neuroscience clinical data: A geneticist's perspective.

Discussion

S. Potkin, University of California, Irvine: The value of big neuroscience clinical data: A neuroimager's perspective.

Discussion

SESSION 2: Breakout Groups

Goal: Discuss What This Precompetitive Neuroscience Data-Sharing Consortium Might Do

Builds on ideas from the morning session and other ideas that participants may have.

Breakout Group I
Leader: R. Conley, Eli Lilly & Company, Indianapolis, Indiana
Rapporteur: H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island

Breakout Group II
Leader: S. Potkin, University of California, Irvine
Rapporteur: M. Schatz, Cold Spring Harbor Laboratory

Breakout Group III
Leader: S. Romano, Pfizer, New York
Rapporteur: J. Sum, First Manhattan Company, New York

SESSION 3: Breakout Groups

Goal: Prioritize in Small Groups What This Precompetitive Neuroscience Data-Sharing Consortium Might Do Prioritization should be based on value and doability of the idea.

Switch Groups: Discuss and Build on Ideas from Other Groups

Leaders: R. Conley, S. Potkin, S. Romano
Rapporteurs: H. Heimer, M. Schatz, Joseph Sum

A. Holden, Pharmaceutical Biomedical Research, Chicago, Illinois: Ongoing Consortium Initiatives: Goals, Successes, Hurdles, Solutions, and Failures: Lessons from the Serious Adverse Event Consortium

Group Discussion

PLENARY SESSION 4: Presentation of Final Ideas and Voting on Priorities of Ideas Consortium

Chairperson: A. Vogt, Hoffmann La-Roche, Basel, Switzerland
Goal: Agree as a Plenary Group on Priorities for What This Precompetitive Neuroscience Data-Sharing Consortium Might Do
Rapporteur: H. Heimer, E. Garofalo, A. Satlin

Group Discussion and Consensus on Objectives for Direction of Consortium

PLENARY SESSION 5: Identifying and Addressing Hurdles to the Development of a Neuroscience Consortium

Goal: Learn from Persons Who Have Led Other Consortia Experienced in Bringing Similar Databases Together

D. Stephenson, Critical Path Institute, Tucson, Arizona: Ongoing consortium initiatives: Goals, successes, hurdles, solutions and failures: Lessons from CAMD.

Group Discussion

J. Rabinowitz, Bar-Ilan University, Ramat-Gan, Israel: Ongoing consortium initiatives: Goals, successes, hurdles, solutions and failures: Lessons from IMI New Med.

Group Discussion

Goal: Focus on Specific Important Hurdles and Their Solutions for Developing This Precompetitive Neuroscience Data-Sharing Consortium

J. Contreras, American University, Washington, DC: Hurdles and solutions: Legal considerations/financial models.

Group Discussion

M. Schatz, Cold Spring Harbor Laboratory: Hurdles and solutions: IT considerations.

Group Discussion

PLENARY SESSION 6

Goal: Review and Build on Ideas from April 29 on the Precompetitive Neuroscience Data-Sharing Consortium. Firmly determine if there will be further work on this effort and, if so, what the next steps will be.

L. Alphs, Janssen Pharmaceuticals, Titusville, New Jersey: Review of Day 1.

Identification of Key Consideration for Development of Precompetitive Neuroscience Data-Sharing Consortium

Mission Statement and Primary Goals

J. Sum, First Manhattan Co., New York: Financial considerations and solutions.

Next Steps

Group Discussion

Organizational Structure and Governance Considerations and Solutions
Receipt and Safety of Data
Analysis of Data
Interpretation of Data
Access to Data

Group Discussion: Identification of outstanding issues and new ideas

Immediate Next Steps and Final Summarization

Redesigning Photosynthesis: Identifying Opportunities and Novel Ideas

May 13–16

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **S. Merchant,** University of California, Los Angeles
D. Ort, University of Illinois, Urbana

D. Ort, S. Merchant

Nearly all other biological processes on earth depend on the ability of photosynthesis to convert solar energy into chemical energy. There is a great deal of interest in the efficiency with which photosynthesis can accomplish this as it is the basis of the yield potential of both our food and bioenergy crops. Sometimes it is stated that photosynthesis is nearly 100% efficient because under ideal conditions, one photon of light can result in one photosynthetic charge separation. But in the world's best agricultural regions, only about 1% of the total solar energy that falls on the field during the growing season is stored as chemical energy in the plant materials at the end of the season. The key question discussed at this meeting was Can the efficiency of solar energy captured by photosynthesis be improved even though evolution has provided very little genetic variation in the component mechanisms of photosynthesis?

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
Workshop Introduction: D. Ort, University of Illinois, Institute for Genomic Biology, Urbana

The purpose of the short (10-min, four-slide) presentations was for the participants to introduce their relevant expertise to the other participants and provide a sense of their research questions, as well as their initial thoughts or ideas toward redesigning photosynthesis. Participants were asked to focus on their work and ideas that were relevant to the workshop and its goals.

Participant Presentations I

J. Alric, CEA Cadarache, Saint-Paul-les-Durance, France
A. Barkan, University of Oregon, Eugene
R. Croce, VU University Amsterdam, The Netherlands
M. Hanson, Cornell University, Ithaca, New York
J. Hibberd, University of Cambridge, England
D. Lindstrom, Agilent Laboratories, Santa Clara, California

Participant Presentations II

S. Merchant, University of California, Los Angeles
T. Moore, Arizona State University, Tempe
J. Moroney, Louisiana State University, Baton Rouge
K. Niyogi, University of California, Berkeley
D. Ort, University of Illinois, Institute for Genomic Biology, Urbana
M. Parry, Rothamsted Research Ltd., Hertfordshire, United Kingdom
P. Peralta-Yahya, Georgia Institute of Technology, Atlanta, Georgia
R. Prince, Exxon Mobil Research and Engineering Co., Annandale, New Jersey
K. Redding, Arizona State University, Tempe
M. Spalding, Iowa State University, Ames

Participant Presentations III

K. Van Wijk, Cornell University, Ithaca, New York
W. Vermaas, Arizona State University, Tempe
T. Yeates, University of California, Los Angeles
J. Yuan, Texas A&M University, College Station
X. Zhu, Chinese Academy of Sciences, Shanghai, China

Sectional Topic Overviews

These longer (25-min) presentations were intended to introduce and give an overview of the opportunities in the different subprocesses or components of photosynthesis.

S. Long, University of Illinois at Urbana-Champaign, Urbana, Illinois: Identifying limitations.
R. Blankenship, Washington University, St. Louis, Missouri: Optimizing/redesigning light capture.
S. von Caemmerer, Australian National University, Canberra: Optimizing/redesigning carbon reduction.
A. Weber, Heinrich–Heine University, Dusseldorf, Germany: Defeating oxygenation/improving photorespiration.
R. Bock, Max-Planck Institute of Molecular Plant, Potsdam-Golm, Germany: Synthetic biology and new tools.

Breakout Groups

Synthesis Session I: Report Back from Breakouts on Priorities for Each Goal. Reorganize breakout groups.

Synthesis Session II: Report Back from Breakouts on Priorities for Each Goal. Discussion of meeting outcomes.

Perspective from a Funding Source

K. Kahn, Bill & Melinda Gates Foundation, Seattle, Washington

Discussion of Next Steps (e.g., Publication, Proposal Initiatives)

S. von Caemmerer, B. Stillman

The Emerging Intersection between Physical Sciences and Oncology

July 14–16

FUNDED BY **USC NCI Physical Sciences in Oncology Center**

ARRANGED BY **D. Agus,** University of Southern California, Los Angeles
D. Hillis, Applied Minds, Inc., Glendale, California
P. Mallick, Stanford School of Medicine, California

This was the second occasion on which the Physical Sciences in Oncology Center came to Banbury Center to report on progress and to stimulate ideas about the challenges and solutions in the detection and treatment of cancer. As before, participants were not restricted to cancer research but included some of the foremost leaders and emerging scientists in clinical care, cancer biology, engineering, and physics. The meeting was structured to promote interactions between members of different research areas by classifying participants into two groups: Group A had a biological/clinical focus and Group B had a technology/engineering focus. Members of each group were paired with a member of the other group to identify a research project of mutual interest and a potential approach for solving it. One objective was to give junior investigators an opportunity to work with more senior investigators and get direct mentorship on how to overcome the challenges associated with working in this highly interdisciplinary field.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Teams

1. J. Iwasa, D. Ruderman, C. Behroozi
2. J. Sachs, A. Sharif, P. Newton
3. P. Bhatnagar, P. Macklin
4. S. Mumenthaler, M. Said, M. Padi
5. H. Karnofsky, V. Stodden, A. Naeim
6. R. Dror, D. Felsher
7. R. Judson, J. LaBaer
8. J. Mogil, P. Mallick, M. Gross

SESSION 1: Presentation of Team 1

SESSION 2: Presentations of Teams 2 and 3

SESSION 3: Presentations of Teams 4 and 5

SESSION 4: Presentations of Teams 6, 7, and 8

SESSION 5: Presentations by Teams 1 and 2

SESSION 6: Presentations by Teams 3 and 4

SESSION 7: Presentations by Teams 5, 6, 7, and 8

Telomeres and Disease

September 8–11

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M. Armanios,** Johns Hopkins University, Baltimore, Maryland
P. Lansdorp, University of Groningen, University Medical Centre Groningen, The Netherlands

A growing body of evidence is implicating telomeres in the pathogenesis of several important and common disorders, including pulmonary fibrosis, bone marrow failure, and diabetes. However, the underlying role of telomeres in these diverse disorders is not fully understood. This discussion meeting brought scientists and clinicians together to review and critically assess current data on how telomere dysfunction contributes to disease. Participants included scientists working on telomere biology as well as in other areas that are relevant to the study of these disorders. The goal was to forge new links between fundamental biology and telomere-mediated disorders.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Overviews: M. Armanios, Johns Hopkins University, Baltimore, Maryland;
P. Lansdorp, University of Groningen, University Medical Centre Groningen, The Netherlands

SESSION 1: Telomerase, Dyskeratosis Congenita, and Mouse Models

Chairperson: L. Harrington, University of Montreal, Quebec, Canada

C. Greider, Johns Hopkins University School of Medicine, Baltimore, Maryland: Short telomeres and mouse models of telomere-mediated disease.

I. Dokal, Barts & The London School of Medicine & Dentistry, London, United Kingdom: Dyskeratosis congenita and related diseases.

M. Bessler, Children's Hospital of Philadelphia, Pennsylvania: Using IPCs for the investigation of X-linked dyskeratosis congenita.

SESSION 2: Telomerase Structure, Function, and Biogenesis

Chairperson: S. Artandi, Stanford University Medical Center, California

K. Collins, University of California, Berkeley: Telomerase holoenzyme regulation.

P. Baumann, Stowers Institute for Medical Research, Kansas City, Missouri: Telomerase RNA biogenesis.

J. Chen, Arizona State University, Tempe: New mechanistic insights into the telomerase catalytic cycle.

SESSION 3: Telomere End-Protection, CST, and Disease

Chairperson: A. Bertuch, Baylor College of Medicine, Houston, Texas

V. Lundblad, Salk Institute for Biological Studies, La Jolla, California: Faithful replication of duplex telomeric DNA is necessary for telomere homeostasis.

T. Linnankivi, University of Helsinki, Finland: The clinical phenotype of Coats plus (CRMCC) syndrome.

E. Jenkinson, University of Manchester, England: Mutations in CTC1, encoding conserved telomere maintenance component 1, cause Coats plus.

C. Price, University of Cincinnati, Ohio: The multiple roles of human CST and how they may relate to human disease.

SESSION 4: DC and Bone Marrow Failure Syndromes II

Chairperson: K. Collins, University of California, Berkeley

A. Bertuch, Baylor College of Medicine, Houston, Texas: Mutations associated with very short leukocyte telomere length and early childhood disease presentation.

S. Savage, National Cancer Institute, Rockville, Maryland: Clinical and epidemiological considerations in telomere biology disorders.

J. Tolar, University of Minnesota, Minneapolis: Refining hematopoietic cell transplantation in dyskeratosis congenita: Where now, and where next?

A. Smogorzewska, Rockefeller University, New York: Fanconi anemia: DNA repair and bone marrow failure syndrome.

SESSION 5: Telomeres and Pulmonary Fibrosis

Chair: C. Price, University of Cincinnati, Cincinnati, Ohio

M. Armanios, Johns Hopkins University, Baltimore, Maryland: Telomeres and age-related lung disease.

B. Hogan, Duke University Medical Center, Durham, North Carolina: Stem cells in the adult lung and models of pulmonary fibrosis.

SESSION 6: Telomeres and Stem Cells

Chairperson: J. Sedivy, Brown University, Providence, Rhode Island

P. Lansdorp, University of Groningen, University Medical Centre, The Netherlands: Mortal and immortal stem cells.

S. Artandi, Stanford University Medical Center, California: Telomerase in stem cells and disease.

R. Reddel, Children's Medical Research Institute, Westmead, Australia: Functional role of ATRX deficiency in ALT

SESSION 7: Senescence and the DNA-Damage Response

Chairperson: A. Smogorzewska, Rockefeller University, New York

J. Sedivy, Brown University, Providence, Rhode Island: How are telomeres, cellular senescence, transposable elements and aging connected?

V. Lundblad, L. Harrington

M. Bessler, I. Dokal

F. D'Adda Di Fagagna, IFOM Foundation–FIRC Institute of Molecular Oncology Foundation, Milan, Italy: Telomeres in aging and cancer.

E. Hendrickson, University of Minnesota Medical School, Minneapolis: Escape from telomere-driven crisis is DNA ligase III-dependent.

L. Harrington, University of Montreal, Quebec, Canada: Latent implications of critically short telomeres on cellular differentiation in aging and disease.

E. Lazzerini Denchi, Scripps Research Institute, La Jolla, California: Shelterin complex mutations and genomic instability.

Conference Room

Neurobiology and Clinical Study of Rapid-Acting Antidepressants

September 15–18

FUNDED BY **Janssen Pharmaceutical Research and Development, Johnson & Johnson**

ARRANGED BY **R. Duman,** Yale University, New Haven, Connecticut
C. Zarate, National Institute of Mental Health, Bethesda, Maryland

Mood disorders affect millions of people worldwide, but a major limitation of existing pharmacotherapies is that they take weeks or months to show therapeutic effects. This lag exerts a toll on patients' well-being and ability to function and increases the already high risk of suicide. Therefore, rapid-onset pharmacological strategies with pronounced and sustained effects would have an enormous impact on public health. Recent studies have found that the drug ketamine produces antidepressant and antisuicidal effects within hours in treatment-resistant depressed patients. However, ketamine also produces psychotic-like symptoms, which limits its therapeutic use. A vigorous effort, both preclinical and clinical, has arisen to explore ketamine's mechanism of action, with an eye toward developing safer alternatives. This meeting provided an opportunity to examine the critical questions and outline the steps to developing safe rapid-acting antidepressants.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: R. Duman, Yale University, New Haven, Connecticut
C. Zarate, National Institute of Mental Health, Bethesda, Maryland

SESSION 1: Rapid Antidepressant Actions of NMDA Receptor Antagonists

Chairperson: H. Mayberg, Emory University School of Medicine, Atlanta, Georgia

J. Krystal, Yale University School of Medicine, New Haven, Connecticut: Overview of the clinical actions of ketamine and glutamate neurobiology.

D. Charney, Icahn School of Medicine at Mount Sinai, New York: Rapid clinical actions of ketamine.

S. Mathew, Baylor College of Medicine, Houston, Texas: Impact of ketamine on suicidality in patients with treatment-resistant depression.

C. Zarate, National Institute of Mental Health, Bethesda, Maryland: Clinical effects of ketamine and biomarkers of treatment response.

W. Drevets, Janssen Research & Development, Titusville, New Jersey: NR2B antagonists as rapid acting antidepressants.

General Discussion

SESSION 2: Rapid Actions of Muscarinic Receptor Antagonists: Mechanisms for Scopolamine and Ketamine

Chairperson: J. Krystal, Yale University School of Medicine, New Haven, Connecticut

M. Furey, National Institute of Mental Health, Bethesda, Maryland: Clinical actions of scopolamine and biomarkers of response.

C. Jones, Vanderbilt University, Nashville, Tennessee: Neurobiology and pharmacology of muscarinic receptors.

G. Sanacora, Yale University School of Medicine, New Haven, Connecticut: Regulation of glutamate/GABA metabolism by rapid antidepressants.

R. Duman, Yale University School of Medicine, New Haven, Connecticut: Synaptogenic actions of rapid-acting antidepressants.

General Discussion

SESSION 3: Neurobiology and Circuitry of Depression and Potential Rapid Antidepressant Targets

Chairperson: R. Duman, Yale University School of Medicine, New Haven, Connecticut

E. Nestler, Icahn School of Medicine at Mount Sinai, New York: Transcriptional and epigenetic mechanisms of depression.

R. Hen, College of Physicians & Surgeons, New York: Hippocampal-amygdala and related circuits in depression and anxiety.

M.-H. Han, Icahn School of Medicine at Mount Sinai, New York: VTA dopamine system and depression.

E. Castren, University of Helsinki, Finland: Isoflurane as a rapid-acting antidepressant.

G. Chen, Johnson & Johnson Pharmaceutical Research and Development, San Diego, California: Preclinical models of depression and drug development.

General Discussion

SESSION 4: Novel Rapid Antidepressant Approaches I: Glutamatergic Mechanisms and Targets

Chairperson: E. Nestler, Icahn School of Medicine at Mount Sinai, New York

D. Bredt, Johnson & Johnson Pharmaceutical Research & Development, San Diego, California: Overview of glutamatergic receptor synaptic mechanisms.

J. Witkin, Lilly Research Laboratories, Indianapolis, Indiana: AMPA receptor potentiation: Potential impact on TRD.

E. Nestler

H. Mayberg

J. Moskal, Northwestern University, Evanston, Illinois: GLYX-13, a novel NMDA receptor modulator with rapid onset and long-lasting antidepressant effects in humans without ketamine-like side effects.
S. Chaki, Taisho Pharmaceutical Co, Ltd., Saitama, Japan: mGlu2/3 receptor antagonists as antidepressants.
P. Skolnick, National Institute on Drug Abuse, Bethesda, Maryland: AMPA receptor potentiators as antidepressants.

General Discussion

SESSION 5: Novel Rapid Antidepressant Approaches II: Drug Development and Clinical Study Design

Chairperson: C. Zarate, National Institute of Mental Health, Bethesda, Maryland

H. Mayberg, Emory University School of Medicine, Atlanta, Georgia: DBS as a rapid antidepressant treatment and neurobiological mechanisms.
W. Bunney, University of California, Irvine: Circadian rhythms, clock genes, and sleep deprivation therapy in depression.
J. Heemskerk, National Institute of Mental Health, Bethesda, Maryland: Funding drug development.
M.R. Trivedi, University of Texas Southwestern Medical Center, Dallas: Integrating biomarkers in clinical research.
M. Fava, Massachusetts General Hospital, Boston: Study design and outcome measures for the NIMH RAPID studies.

Closing Discussion and Summary

Plant Reproduction

September 22–25

FUNDED BY **CSHL/DuPont Pioneer Joint Collaborative**

ARRANGED BY **R. Martienssen,** Cold Spring Harbor Laboratory
R. Meeley, DuPont Pioneer, Johnston, Iowa

This was the annual meeting of the collaborative project between the plant science group at Cold Spring Harbor Laboratory and scientists at DuPont Pioneer. The goals of this meeting were to explore the latest advances in our understanding of sexual and asexual reproduction in crop and model plant species and to drive discussions on current research addressing genetic, epigenetic, and population-based approaches to manipulating key mechanisms in plant reproductive biology. As usual, one day was devoted to presentations from speakers outside the collaboration.

Welcoming Remarks: J.A Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: R. Meeley, DuPont Pioneer, Johnston, Iowa
R. Martienssen, Cold Spring Harbor Laboratory

SESSION 1: Data Mining in Expression Networks

Chairperson: R. Meeley, DuPont Pioneer, Johnston, Iowa

Y.K. Lee, Cold Spring Harbor Laboratory: The *Arabidopsis* root miRNA regulatory network supports functional characterization of transcription factors involved in development and environmental response.

C. Liseron-Monfils, Cold Spring Harbor Laboratory: The dynamic of gene coexpression within *Arabidopsis* miRNA-based genetic network during plant development and response to stresses.

A. Eveland, Cold Spring Harbor Laboratory: Systems approaches in maize inflorescence architecture and drought.

SESSION 2: Somatic Patterning and Small RNA

Chairperson: M. Cigan, Pioneer Hi-Bred International, Johnston, Iowa

K. Petsch, Cold Spring Harbor Laboratory: Hierarchical *DICER* activity in maize triggers alternate processing of tasiARF target transcripts.

M. Timmermans, Cold Spring Harbor Laboratory: Patterning properties of mobile small RNAs.

Y. Plavskin, Cold Spring Harbor Laboratory: Regulation of the auxin response by an ancient small RNA pathway.

SESSION 3: Genetic Dissection of Inflorescence Development

Chairperson: L. Perugini, DuPont Pioneer, Johnston, Iowa

B. ll Je, Cold Spring Harbor Laboratory: Finding the function of *fasciated ear3.*

M. Pautler, Cold Spring Harbor Laboratory: *FASCIATED EAR4:* Function and targets.

D. Jackson, Cold Spring Harbor Laboratory: Update on *RAMOSA3* in maize and *Arabidopsis.*

SESSION 4: Targeted Molecular and Genetic Strategies

Chairperson: D. Jackson, Cold Spring Harbor Laboratory

L. Perugini, DuPont Pioneer, Johnston, Iowa: Characterization of ear trait mutants for increasing yield in elite maize germplasm.

B. Li, DuPont Experimental Station, Wilmington, Delaware: Understanding the molecular mechanisms of gene–background interactions: Two case studies.

M. Cigan, Pioneer Hi-Bred International, Johnston, Iowa: Targeted genome modification of plant fertility genes using double-strand-break reagents.

M. Williams, DuPont Experimental Station, Wilmington, Delaware: The interaction of genetics and mutagenesis.

SESSION 5: A Landscape of Reproductive Strategies

Chairperson: S. Lawit, DuPont Pioneer, Johnston, Iowa

M. Singh, DuPont Pioneer, Johnston, Iowa: Genetic and epigenetics of apomixis in maize.

A. Schnittger, Institut de Biologie Moleculaire des Plantes, Strasbourg, France: Control of germline entry in *Arabidopsis.*

T. Dresselhaus, Universitat Regensburg, Germany: Fertilization mechanisms and early embryogenesis in maize.

SESSION 6: Developmental and Reproductive Outcomes of Auxin Signaling

Chairperson: B. Li, DuPont Pioneer, Wilmington, Delaware

P. McSteen, University of Missouri, Columbia: Role of auxin in maize inflorescence development.

M. Evans, Carnegie Institution for Science, Stanford, California: Mutant analysis of maize antipodal cells and auxin signaling.

SESSION 7: Epigenetics that Pattern Reproductive Boundaries

Chairperson: M. Singh, DuPont Pioneer, Johnston, Iowa

C. Kohler, Swedish University of Agriculture Science, Uppsala, Sweden: Epigenetic mechanisms establishing interploidy and interspecies hybridization barriers in the endosperm.

R. Martienssen, Cold Spring Harbor Laboratory: Reprogramming heterochromatin in the germline and its consequences.

SESSION 8: Technical Approaches to Recombination

Chairperson: R. Martienssen, Cold Spring Harbor Laboratory

G. May, DuPont Pioneer, Johnston, Iowa: Genomic approaches to recombination.

W. Pawlowski, Cornell University, Ithaca, New York: The landscape of meiotic recombination in maize.

A. Eveland

B. Li, D. Jackson

R. Mercier, INRA Centre de Versailles-Grignon, Versailles, France: What limits meiotic recombination?

F. de Sousa Borges, Cold Spring Harbor Laboratory: The pollen methylome and implications for epiallele formation.

SESSION 9: Genetic and Epigenetic Pathways in Flowering and Reproductive Success

Chairperson: M. Komatsu, DuPont Pioneer, Wilmington, Delaware

Z. Lippman, Cold Spring Harbor Laboratory: Fine-tuning flowering to boost yield.

C. Xu, Cold Spring Harbor Laboratory: The TMF-BOP complex directs inflorescence architecture in tomato.

C. MacAlister, Cold Spring Harbor Laboratory: From meristems to pollen tubes to protonema: An unknown gene family with diverse functions in plant development.

SESSION 10: Development of Apomictic Strategies

Chairperson: M. Timmermans, Cold Spring Harbor Laboratory

R. Herridge, Cold Spring Harbor Laboratory: The role of argonautes in reproductive strategies in *Arabidopsis.*

S. Lawit, DuPont Pioneer, Johnston, Iowa: Research frontiers of plant female reproductive strategies in *Arabidopsis.*

M. Williams, DuPont Stine-Haskell Research Center, Newark, Delaware: Self-reproducing hybrid technology in maize.

Meeting Wrap-Up

Science of Pancreatic Cancer

September 29–October 1

FUNDED BY **MCJ Amelior Foundation and Kotumba Capital Management LLC**

ARRANGED BY **R. Evans,** Salk Institute for Biological Studies, La Jolla, California
W. Isacoff, University of California, Los Angeles
D. Tuveson, Cold Spring Harbor Laboratory

Recent findings in pancreatic cancer science and medicine demonstrate that both neoplastic cell genetic changes and distinct features of the tumor microenvironment may serve as therapeutic vulnerabilities in this malignancy. This meeting focused on the role of the stroma in modulating therapeutic responses and the development of new dependency pathways. Topics reviewed included vitamin D and pancreatic stellate cell activation; survival cues in the tumor microenvironment as major causes of drug resistance; methods to develop a tissue bank of the tumor microenvironment and cancer cells; and the role of genomics in addressing this disease. The meeting concluded with a discussion intended to help identify one or two important areas worthy of large-scale additional investigation.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Pancreatic Stromal Cells and Opportunities

Chairperson: D. Tuveson, Cold Spring Harbor Laboratory

R. Kalluri, University of Texas, MD Anderson Cancer Center, Houston: Stromal biology in PDAC.

D. Fearon, CRUK Cambridge Institute, United Kingdom: FAP+ cells and immune suppression.

M. Egeblad, Cold Spring Harbor Laboratory: Imaging the PDAC stroma.

SESSION 2: Pancreatic Stroma and Manipulation

Chairperson: D. Tuveson, Cold Spring Harbor Laboratory

R. Evans, Salk Institute for Biological Studies, La Jolla, California: PSCs and vitamin D.

M. Hollingsworth, University of Nebraska Medical Center, Omaha: The best targets in PDAC.

G. Wahl, Salk Institute for Biological Studies, La Jolla, California: Stromal and epithelial approaches to pancreatic cancer.

General Discussion

SESSION 3: Neoplastic Cells and Therapeutic Opportunities in PDAC

Chairperson: R. Evans, Salk Institute for Biological Studies, La Jolla, California

T. Hunter, Salk Institute for Biological Studies, La Jolla, California: Secreted proteins that mediate cross-talk between stellate cells and tumor cells.

A. Maitra, University of Texas, MD Anderson Cancer Center, Houston: Triple metabolism therapy in PDAC.

K. Olive, Columbia University, New York: Targeting ROS detoxification in pancreatic cancer.

SESSION 4: Neoplastic Cells and Therapeutic Opportunities

Chairperson: R. Evans, Salk Institute for Biological Studies, La Jolla, California

A. Lowy, University of California, San Diego, Moores Cancer Center, La Jolla, California: Targeting RON in PDAC.

H. Crawford, Mayo Clinic Florida, Jacksonville, Florida: Signaling cascades as targets in PDAC.

S. Muthuswamy, University of Toronto, Ontario, Canada: A new model system for PDAC.

Summary Discussion, What Have We Heard?

P. Philip, Karmanos Cancer Center, Detroit, Michigan

B. Stillman, Cold Spring Harbor Laboratory

SESSION 5: Additional Opportunities in PDAC

Chairperson: W. Isacoff, University of California, Los Angeles

C. Iacobuzio-Donahue, Johns Hopkins University, Baltimore, Maryland: Moving genetic targets in PDAC.

N. Bardeesy, Massachusetts General Hospital Cancer Center, Boston: Role of MiT proteins in metabolic reprogramming in pancreatic cancer.

S. Leach, Johns Hopkins University, Baltimore, Maryland: Targeting PanIN initiation and progression.

SESSION 6: Final Discussion and Next Steps

Chairperson: W. Isacoff, University of California, Los Angeles

P. Philip, Karmanos Cancer Institute, Detroit, Michigan: Status of clinical trials.

T. Donahue, David Geffen School of Medicine, Los Angeles, California: Translational PET imaging to guide chemotherapy in human pancreatic cancer.

Final Meeting Summary: Is the Science Ready for Transformative Clinical Efforts?

W. Isacoff, University of California, Los Angeles

R. Evans, Salk Institute for Biological Studies, La Jolla, California

D. Tuveson, Cold Spring Harbor Laboratory

T. Hunter

Biguanides and Neoplasia

October 6–9

FUNDED BY **Oliver Grace Cancer Fund**

ARRANGED BY **M. Pollak,** McGill University, Montreal, Quebec, Canada
K. Struhl, Harvard Medical School, Boston, Massachusetts

Interest in potential roles of biguanides such as metformin in treatment and/or prevention of neoplastic disease continues to increase since the topic was last discussed at Banbury in 2011. Participants in the 2013 meeting discussed the nature of the primary site of action in mitochondria, the alterations in cellular energetics and metabolism caused by biguanides, and the genetic factors that influence these effects. Additionally, the effects of biguanides at the whole-organism level were reviewed, including modulation of both inflammatory responses and the endocrine environment. An important discussion centered on strategies for optimizing drug exposure to target tissues, which may differ from those important in diabetes treatment. Finally, the use of preclinical findings to optimize the design of future trials was reviewed.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: K. Struhl, Harvard Medical School, Boston, Massachusetts

Overview of Progress Since Last Meeting: M. Pollak, McGill University, Montreal, Quebec, Canada

SESSION 1

Chairperson: M. Pollak, McGill University, Montreal, Quebec, Canada

J. Hirst, The Medical Research Council, The Wellcome Trust/MRC Building, Cambridge, United Kingdom: Effects of biguanides on mitochondrial complex I.

M. Schwab, University Hospital of Tuebingen, Stuttgart, Germany: Metformin and drug disposition: Update and future perspectives.

B. Kahn, Beth Israel Deaconess Medical Center, Boston, Massachusetts: AMPK and the regulation of food intake, body weight, and metabolism.

SESSION 2

Chairperson: K. Struhl, Harvard Medical School, Boston, Massachusetts

L. Cantley, Weill Cornell Medical College, New York: AMPK and cancer.

R. Shaw, Salk Institute for Biological Studies, La Jolla, California: LKB1/STK11 genotype dictates therapeutic response to phenformin.

J. Pouyssegur, University of Nice, France: Targeting glycolysis (lactate transporters) sensitizes tumor cells to phenformin.

M. Pollak, McGill University, Montreal, Canada: Serine deficiency sensitizes neoplastic cells to phenformin.

K. Birsoy, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Metabolic liabilities of cancer cells to nutrient limitation.

SESSION 3

Chairperson: R. Shaw, Salk Institute for Biological Studies, La Jolla, California

M. Stampfer, Harvard University, Brigham and Women's Hospital, Boston, Massachusetts: Metformin and prostate cancer prevention.

N. Fleshner, Princess Margaret Hospital, Toronto, Canada: Rationale for metformin in prostate cancer.

C. Dang, University of Pennsylvania, Philadelphia: Activities of biguanides and metabolic inhibitors in human pancreatic cancer xenografts.

N. Hay, University of Illinois, Chicago: Targeting glucose metabolism for cancer therapy

P. Puigserver, Dana-Farber Cancer Institute, Boston, Massachusetts: Therapeutic implications of metabolic and energy flexibility in melanoma tumors.

M. Keiser, SeaChange Pharmaceuticals, Inc. San Francisco, California: Prediction and testing of a new target for metformin with a potential role in neoplasia.

H. Udono, Okayama University, Japan: Metformin-induced reversion of immune-exhaustion in tumor microenvironment.

SESSION 4

Chairperson: R. Jones, McGill University, Montreal, Quebec, Canada

J. Schlessinger, Yale University, New Haven, Connecticut: Targeting receptor tyrosine kinases.

K. Struhl, David Geffen School of Medicine, Los Angeles, California: Metformin mediates anticancer effects by inhibiting the inflammatory pathway.

J.D. Watson, Cold Spring Harbor Laboratory: Exercise vs. metformin.

K. Vousden, Beatson Institute, Glasgow, United Kingdom: Regulation of metabolism through the p53 pathway.

G. Thomas, University of Cincinnati, Ohio: Metformin in the treatment of HCC?

N. Sonenberg, McGill University, Montreal, Quebec, Canada: Translational control of mitochondria function via mTOR.

M. Pollak, F. Cabreiro, N. Sonenberg, K. Vousden

J. Hirst

R. Kalluri, MD Anderson Cancer Center, Houston, Texas: Designing rational preclinical combination trials for pancreatic cancer (PDAC).

SESSION 5

Chairperson: K. Vousden, Beatson Institute, Glasgow, United Kingdom

P. Dennis, Johns Hopkins Bayview Medical Center, Baltimore, Maryland: Mechanisms of chemoprevention by metformin.

B. Zheng, Harvard Medical School, Charlestown, Massachusetts: Targeting AMPK signaling in melanoma.

G. Ferbeyre, University of Montreal, Canada: Metformin and the NF-κB pathway.

M. VanderHeiden, Massachusetts Institute of Technology, Cambridge: Understanding tumor metabolism in vivo: Implications for use of metformin to treat cancer.

F. Cabreiro, University College London, United Kingdom: Biguanides regulate microbial function to modulate host health and lifespan.

Lustgarten Foundation Scientific Meeting

October 21–22

FUNDED BY **Lustgarten Foundation for Pancreatic Cancer, Bethpage, New York**

ARRANGED BY **M. McCurragh,** Lustgarten Foundation for Pancreatic Cancer, Bethpage, New York

This meeting provided an opportunity for investigators supported by the Lustgarten Foundation to meet and to present and discuss their research. The goals of the meeting were to update the Lustgarten Foundation research community of progress in the laboratory, to evaluate performance and provide feedback for improvement, and to establish and strengthen collaborations between groups and brainstorm new ideas to push the field forward.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: R. Vizza, Lustgarten Foundation for Pancreatic Cancer, Bethpage, New York
D. Tuveson, Cold Spring Harbor Laboratory

Key Note Speaker: B. Vogelstein, Johns Hopkins University School of Medicine, Baltimore, Maryland

PRECLINICAL GROUP

H.C. Manning, Vanderbilt University Institute of Imaging Science, Nashville, Tennessee
Q. Nguyen, University of California, San Diego, La Jolla
S. Thorne, University of Pittsburgh, Pennsylvania
S. Hingorani, Fred Hutchinson Cancer Research Center, Seattle, Washington

General Discussion

Keynote Speaker: Cold Spring Harbor Laboratory

CONSORTIUM PANEL 1

S. Lowe, Memorial Sloan-Kettering Cancer Center, New York

C. Castro, Harvard University Medical School, Boston, Massachusetts
B. Wolpin, Dana Farber Cancer Institute, Boston, Massachusetts

General Discussion

BASIC GROUP 1

D. Fearon, CRUK Cambridge Institute, United Kingdom
G. Miller, New York University Langone Medical Center
E. O'Reilly, Memorial Sloan-Kettering Cancer Center, New York

General Discussion

BASIC GROUP 2

D. Bar-Sagi, New York University School of Medicine, New York
A. Kimmelman, Dana-Farber Cancer Institute, Boston, Massachusetts
M. Egeblad, Cold Spring Harbor Laboratory

General Discussion

BASIC GROUP 3

J.J. Yeh, University of North Carolina, Chapel Hill
T. Wang, Columbia University, New York

General Discussion

CLINICAL GROUP

H. Degani, Weizmann Institute of Science, Rehovot Israel
J. Fleming, MD Anderson Cancer Center, Houston, Texas

General Discussion

CONSORTIUM PANEL 2

C. Der, University of North Carolina, Chapel Hill
T. Van Dyke, Frederick National Laboratory for Cancer, Frederick, Maryland
S. Fesik, Vanderbilt University School of Medicine, Nashville, Tennessee

General Discussion

Meeting Summary and Future Goals
D. Tuveson, Cold Spring Harbor Laboratory

J. Watson, D. Tuveson, R. Vizza

Q. Nguyen

Ovarian Cancer: Developing Research-Based Public Messaging on Early Detection and Screening

October 23–25

FUNDED BY **Ovarian Cancer Research Fund**

ARRANGED BY **J. Boyd,** Fox Chase Cancer Center, Philadelphia, Pennsylvania
A. Moran, Ovarian Cancer Research Fund, New York
M. Seiden, McKesson Specialty Health, Woodlands, Texas

M. Seiden, A. Moran

Messages to the public about ovarian cancer should accurately reflect what research results currently demonstrate, as well as what might reasonably be expected in the near term. When the public talks about ovarian cancer and ovarian cancer research, much emphasis is placed on early detection of the disease, as well as symptoms as a means of saving lives. In practice, the matter is more complicated, depending on the type of cancer, the efficacy of the screening, and the consequences of false positives. The UK Collaborative Trial of Ovarian Cancer Screening is under way, designed to provide firm data that can be used as the basis for assessing the value of current methods of early detection of ovarian cancer. The findings of these trials will have a major impact on the ovarian cancer community. This meeting was held to review the current status of ovarian cancer screening, to discuss action that might be taken for either positive or negative results of the UKCTOCS study, and to use these discussions as the basis for developing clearly defined messages that can help lay public understand the implications of the findings.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
A. Moran, Ovarian Cancer Research Fund, New York

Introduction: Defining the Problem, What We Hope to Accomplish
J. Boyd, Fox Chase Cancer Center, Philadelphia, Pennsylvania
M. Seiden, McKesson Specialty Health, Woodlands, Texas

SESSION 1: State of Science

J. Boyd, Fox Chase Cancer Center, Philadelphia, Pennsylvania: Whence epithelial ovarian carcinoma?
N. Urban, Fred Hutchinson Cancer Research Center, Seattle, Washington: Symptoms index and multimodal screening: Using novel markers to improve screening performance.
K. Lu, MD Anderson Cancer Center, Houston, Texas: MD Anderson Study: A stage-2 Ovarian cancer screening strategy using the risk of ovarian cancer algorithm (ROCA) identifies early-stage incident cancers and demonstrates high positive predictive value.
I. Jacobs, University of Manchester, United Kingdom: UKCTOCS update.
U. Menon, University College London, United Kingdom: Time series algorithms: What can we learn from UKCTOCS.
S. Skates, Massachusetts General Hospital, Boston: ROCA: Development and implementation in screening trials.
S. Narod, Women's College Research Institute, Toronto, Canada: Perspectives for screening for ovarian cancer.

SESSION 2: Defining Benefit

K. Trivers, Centers for Disease Control, Atlanta, Georgia: A public health approach to understanding ovarian cancer.
M. Ebell, University of Georgia, Athen, Georgia: The US Preventive Services Task Force and Ovarian Cancer Screening

Group Discussion: Provocative Questions
Moderators: J. Boyd, Fox Chase Cancer Center, Philadelphia, Pennsylvania
M. Seiden, McKesson Specialty Health, Woodlands, Texas

Day-One Recap: Group Discussion
Moderators:
J. Boyd, Fox Chase Cancer Center, Philadelphia, Pennsylvania
M. Seiden, McKesson Specialty Health, Woodlands, Texas

Concluding Discussion

U. Menon, S. Skates

Enhancer Biology in Health and Disease

October 27–30

FUNDED BY **Oliver Grace Cancer Fund**

ARRANGED BY **J. Bradner,** Dana-Farber Cancer Institute, Boston, Massachusetts
J. Wysocka, Stanford School of Medicine, California
R. Young, Whitehead Institute, MIT, Cambridge, Massachusetts

There has been rapid progress in identifying transcriptional regulatory elements and the factors that occupy them. Disease-associated sequence variation occurs in some of these regulatory elements and in the factors that bind them. This meeting brought together experts in enhancer biology to discuss the roles of regulatory elements and factors in control of gene expression programs and their impact on human health and disease.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Enhancers and Chromatin Folding

Chairperson: J. Bradner, Dana-Farber Cancer Institute, Boston, Massachusetts

R. Young, Whitehead Institute, MIT, Cambridge, Massachusetts: Super-enhancers.

B. Ren, University of California, San Diego: Functional relationship between DNA looping and enhancer activities.

J. Dekker, University of Massachusetts, Worcester: Chromosome folding and long-range gene regulation.

M. Merkenschlager, MRC Clinical Sciences Centre, Imperial College London, United Kingdom: Cohesin and the regulation of gene expression.

V. Corces, Emory University, Atlanta, Georgia: The role of architectural proteins in organizing the 3D architecture of the genome.
W. Bickmore, University of Edinburgh, Scotland: Do enhancers function in compact chromatin domains?

General Discussion

SESSION 2: Enhancer Dynamics

Chairperson: E. Furlong, EMBL, Heidelberg, Germany
M. Levine, University of California, Berkeley: Enhancer dynamics in the *Drosophila* embryo.
J. Wysocka, Stanford School of Medicine, California: Enhancer regulation in development.
K. Zaret, Perelman School of Medicine, Philadelphia, Pennsylvania: Creating active enhancers.

SESSION 3: Enhancers in Disease Part 1: Genomic Variation

Chairperson: J. Bradner, Dana-Farber Cancer Institute, Boston, Massachusetts
M. Maurano, University of Washington, Seattle: Regulatory variation and human disease.
S. Parker, National Institutes of Health, Bethesda, Maryland: Stretch enhancers drive cell-specific gene regulation and harbor human disease variants.
P. Scacheri, Case Western Reserve University School of Medicine, Cleveland, Ohio: Combinatorial effects of multiple enhancer variants in common disease.

General Discussion

SESSION 4: Enhancers in Disease Part II: Coactivator Function

Chairperson: R. Young, Whitehead Institute, MIT, Cambridge, Massachusetts
J. Bradner, Dana-Farber Cancer Institute, Boston, Massachusetts: Disrupting enhancer function to discover and downregulate cancer dependencies.
C. Vakoc, Cold Spring Harbor Laboratory: Targeting coactivator proteins in acute myeloid leukemia.

SESSION 5: Large-Scale Functional Analysis

Chairperson: B. Ren, University of California, San Diego
A. Stark, Research Institute of Molecular Pathology, Vienna, Austria: Decoding transcriptional regulatory sequences.
M. Bulyk, Brigham & Women's Hospital, Boston, Massachusetts: Highly parallel enhancer assays in whole *Drosophila* embryos.
B. Bernstein, Broad Institute, Charlestown, Massachusetts: Manipulating *cis*-element landscapes in human cells.

General Discussion

SESSION 6: Enhancer Factors And Function

Chairperson: M. Levine, University of California, Berkeley
J. Zeitlinger, Stowers Institute for Medical Research, Kansas City, Missouri: Dissecting transcription factor binding at *Drosophila* enhancers using ChIP-exo.
G. Crabtree, Stanford University School of Medicine, California: ATP-dependent chromatin remodeling and enhancer function.

J. Dekker, K. Zaret

M. Levine

D. Odom, University of Cambridge, United Kingdom: Insights into mammalian enhancers from comparative functional genomics.

SESSION 7: Developmental Mechanisms

Chairperson: J. Wysocka, Stanford School of Medicine, California

E. Furlong, EMBL, Heidelberg, Germany: Temporal properties of enhancer activity during development.

T. Maniatis, Columbia University Medical Center, New York: Generation of cell surface diversity through stochastic enhancer/promoter interactions.

S. Lomvardas, University of California, San Francisco: Synergistic action of distant enhancers specifies singular olfactory receptor expression.

General Discussion

SESSION 8: Enhancers and Noncoding RNA

Chairperson: M. Bulyk, Brigham & Women's Hospital, Boston, Massachusetts

R. Shiekhattar, Wistar Institute, Philadelphia, Pennsylvania: Biogenesis and mechanism of action of enhancer RNAs.

M.G. Rosenfeld, University of California, San Diego: Nuclear receptor and IncRNA regulation of enhancer function.

J. Rinn, Broad Institute of MIT and Harvard, Cambridge, Massachusetts: Linking RNA to nuclear architecture.

Concluding Discussion: Challenges to Developing Therapies That Target Enhancer Function in Disease

INK4/ARF Network

November 12–15

FUNDED BY **Pfizer, Inc.**

ARRANGED BY **D. Beach,** University of London, United Kingdom
N.E. Sharpless, University of North Carolina, Chapel Hill
C.J. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee

This meeting celebrated the 20th anniversary of the discovery of the *INK4/ARF* locus encoding three tumor suppressor proteins that coordinate signaling of the CDK4/6-retinoblastoma (RB) and MDM2-p53 pathways. Disruption of this circuitry, frequently by deletion or silencing of *INK4/ARF*, is a hallmark of many cancers. The *INK4/ARF* locus may have evolved to physiologically restrict the self-renewal capacities and numbers of stem and progenitor cells with the attendant consequence of limiting tissue regenerative capacity, particularly as animals age. In accord with this concept, altered regulation of the *INK4/ARF* locus has been implicated in age-associated diseases in humans by unbiased, genome-wide analyses. Participants in the meeting reviewed a wide-ranging set of issues relating to the evolution and biology of the locus, and its implications for therapies.

J. Watson, D. Beach, K. Knudsen

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Historical Overview: C. Sherr, St. Jude Children's Research Hospital, Memphis, Tennessee

SESSION 1: Signaling through the RB Pathway

Chairperson: G. Peters, CRUK London Research Institute, United Kingdom

J. Lees, Koch Institute, MIT, Cambridge, Massachusetts: Are Ink4, CycD/K4, and Rb mutations synonymous in tumorigenesis?

J. Sage, Stanford University Medical Center, California: The RB gene family in cell cycle control and cancer.

J.A. Diehl, University of Pennsylvania, Philadelphia: Dysregulation of D-type cyclins in cancer.

J. Bartek, Danish Cancer Society, Copenhagen, Demark: INK4/ARF and the DNA-damage response as a barrier to cancer progression.

General Discussion

SESSION 2: New Approaches to Cancer Treatment

Chairperson: N. Sharpless, Lineberger Comprehensive Center, Chapel Hill, North Carolina

K. Knudsen, Thomas Jefferson University, Philadelphia, Pennsylvania: Translating alterations of the p16/RB locus to the clinic: Prostate cancer.

M. Gillison, Ohio State University, Columbus: p16 and prognosis of head and neck cancer.

G. Shapiro, Harvard Medical School, Boston, Massachusetts: Clinical development of selective CDK4/6 inhibitors.

K. Arndt, Pfizer Worldwide Research & Development, Pearl River, New York: Palbociclib inhibition of CDK4/6 as a treatment for cancer.

General Discussion

SESSION 3: INK4/ARF and Aging

Chairperson: M. Serrano, Spanish National Cancer Research Center, Madrid, Spain

N. Sharpless, University of North Carolina, Chapel Hill: p16, aging, and cancer.

C. Bishop, Barts & The London School of Medicine, London, United Kingdom: p16 and senescence.

J.D. Watson, Cold Spring Harbor Laboratory: RAS, ROS, PTEN, and senescence.

C. Burd, Ohio State University, Columbus: Reporter model for p16 regulation.

J. van Deursen, Mayo Clinic, Rochester, Minnesota: p16-positive senescent cells in aging and age-related disease

SESSION 4: Senescence Networks and Development

Chairperson: J. Sage, Stanford University Medical Center, California

M. Serrano, Spanish National Cancer Research Center, Madrid, Spain: INK4/ARF locus: Developmental senescence and in vivo reprogramming.

S. Lowe, Memorial Sloan-Kettering Cancer Center, New York: New insights into the p53 tumor suppressor networks.

D. Peeper, Netherlands Cancer Institute, Amsterdam, The Netherlands: p16: A smoking gun in melanoma senescence.

S. Skapek, University of Texas Southwestern Medical Center, Dallas: Insights into Arf biology from studying blind mice.

General Discussion

SESSION 5: Regulation of INK4/ARF Gene Expression

Chairperson: J. Lees, Koch Institute, MIT, Cambridge, Massachusetts

G. Peters, CRUK London Research Institute, London, United Kingdom: Polycomb regulation of INK4a.

A. Bracken, Trinity College, Dublin, Ireland: Polycomb regulation of the INK4/ARF locus.

G. Shapiro

J. Lees

J. Gil, MRC Clinical Sciences Centre, London, United Kingdom: Regulation of INK4/ARF by SWI/SNF and other chromatin modifiers.

A. Mills, Cold Spring Harbor Laboratory: Chd5-mediated regulation of the Ink4/Arf tumor suppressor network.

Y. Xiong, University of North Carolina, Chapel Hill: Epigenetic regulation of p16 and Arf.

Closing Remarks

Sammis, Winter 2013

The Adolescent Brain

December 3–5

FUNDED BY **The Allen Institute for Brain Science, the Lieber Institute for Brain Development, the National Institute on Alcohol Abuse and Alcoholism, and the National Institute of Mental Health**

ARRANGED BY **J. Giedd,** National Institute of Mental Health, Bethesda, Maryland
H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island
E. Lein, Allen Institute for Brain Science, Seattle, Washington
N. Sestan, Yale University School of Medicine, New Haven, Connecticut

It has long been noted that many psychiatric disorders first make their appearance in adolescence and the transition to adulthood. Adolescence is a time of great developmental change in the human brain, and it is becoming clear that the origins of at least some of these disorders lie in the failure of normal brain development. Modern neuroscience has revealed a great deal about prenatal and early postnatal human brain development, but it has not provided much detail about later stages of neurodevelopment. This meeting surveyed the state of knowledge about normal adolescent brain and behavior and the apparent special vulnerability of the adolescent brain to mental disorders. The most recent data were critically reviewed with the aim of producing an integrated account that will point out the significant gaps in our knowledge.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Workshop Introduction: H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island

SESSION 1: Overviews

Chairperson: H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island

T. Paus, Rotman Research Institute, University of Toronto, Ontario, Canada: Adolescent brain development.

K. Mirnics, P. Rakic

C. Colantuoni, D. Weinberger

B.J. Casey, Sackler Institute, Weill Cornell Medical College, New York; Adolescent behavioral development.
J. Giedd, National Institute of Mental Health, Bethesda, Maryland: Mental illness in adolescence.
D. Weinberger, Lieber Institute for Brain Development, Baltimore, Maryland: Developmental biology and psychopathology.

SESSION 2: Short Presentations A

Chairperson: N. Sestan, Yale University School of Medicine, New Haven, Connecticut

P. Schmidt, National Institute of Mental Health, Bethesda, Maryland
E. Sowell, University of Southern California, Los Angeles
J. Tollkuhn, University of California, San Francisco
B. Luna, University of Pittsburgh, Pennsylvania
N. Tottenham, University of California, Los Angeles
Z.J. Huang, Cold Spring Harbor Laboratory

Key Issues

SESSION 3: Short Presentations B

Chairperson: E. Lein, Allen Institute for Brain Science, Seattle, Washington
C. Sisk, Michigan State University, East Lansing
R. Gur, Perelman School of Medicine, Philadelphia, Pennsylvania
L. Spear, Binghamton University, New York
I. Gotlib, Stanford University, California
F. Lee, Weill Cornell Medical College, New York

Key Issues

SESSION 4: Short Presentations C

Chairperson: J. Giedd, National Institute of Mental Health, Bethesda, Maryland
P. Rakic, Yale University School of Medicine, New Haven, Connecticut
K. Mirnics, Vanderbilt University, Nashville, Tennessee
C. Colantuoni, Lieber Institute for Brain Development, Baltimore, Maryland
E. Lein, Allen Institute for Brain Science, Seattle, Washington
N. Sestan, Yale University School of Medicine, New Haven, Connecticut
P. Mitra, Cold Spring Harbor Laboratory

Key Issues

SESSION 5: Revisiting Key Issues

Chairperson: J. Giedd, National Institute of Mental Health, Bethesda, Maryland

SESSION 6: Discussion of Next Steps (e.g., Publication, Proposal Initiatives)

Chairperson: N. Sestan, Yale University School of Medicine, New Haven, Connecticut

Psychiatric Genomics: Current Status, Future Strategies

December 8–11

FUNDED BY **The Stanley Research Fund**

ARRANGED BY **W.R. McCombie,** Cold Spring Harbor Laboratory
A. Palotie, University of Helsinki, Finland

It has been difficult to find the genes and gene loci underlying psychiatric and other complex disorders. However, recent GWA studies and new high-throughput DNA sequencing techniques have provided new promise. Although there are good standards and practices to analyze GWAS data, the interpretation and analysis of sequence data are still in their infancy. This meeting brought together experts to critically assess current strategies and to outline how genome-scale sequencing can be used most effectively and efficiently. Topics covered included the following: How can high-throughput sequencing build on GWA studies? How should candidate rare risk alleles be validated? How will we ensure that data will be accessible to the community at large, while protecting the legitimate intellectual concerns of primary investigators, not to mention the privacy concerns of study subjects?

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: W.R. McCombie, Cold Spring Harbor Laboratory
A. Palotie, University of Helsinki, Finland

SESSION 1: What Can We Learn from Genotype-Based Association Studies?

Chairperson: J. Knowles, University of Southern California, Los Angeles

P. Sullivan, University of North Carolina, Chapel Hill: PGC, update on the schizophrenia GWAS.

B. Neale, Harvard University, Boston, Massachusetts: Statistics, and why we need them.

T. Pers, Harvard University, Boston, Massachusetts: Selecting likely causal genes and pathways from GWAS by data integration.

T. Lencz, North Shore LIJ Health System, Glen Oaks, New York: Genetic studies in schizophrenia: Expanding the scope by narrowing the focus.

General Discussion

SESSION 2: What Can We Learn from Sequence-Based Association Studies?

Chairperson: D. Porteous, University of Edinburgh, United Kingdom

S. Purcell, Mount Sinai School of Medicine, New York: Schizophrenia case control exome sequencing association.

D. Porteous

M. Daly, Harvard University, Boston, Massachusetts: Exome sequencing and the genetic architecture of autism spectrum disorders.

L. Scott, University of Michigan, Ann Arbor: Whole-genome and -exome sequencing of bipolar disorder.

D. Goldstein, Duke University, Durham, North Carolina: Lessons from Mendelian genetics in complex neuropsychiatric disease.

General Discussion

SESSION 3: What Can We Learn from Sequencing Families?

Chairperson: D. Goldstein, Duke University, Durham, North Carolina

J. Knowles, University of Southern California, Los Angeles: RNA-Seq: BrainSpan, CNON, and single cells.

D. Porteous, University of Edinburgh, United Kingdom: Generation Scotland GWAS for major depressive disorder and related traits.

A. Corvin, Trinity College, Dublin, Ireland: Common and rare variants implicate PAK signaling in psychosis susceptibility.

M. Wigler, Cold Spring Harbor Laboratory: Gene target discovery in autism by family exome sequencing.

M.-C. King, University of Washington, Seattle: Damaging de novo mutations in schizophrenia: Identification and mapping to prefrontal cortex.

J. McClellan, University of Washington, Seattle: Damaging de novo mutations in schizophrenia: Insights from gene function.

General Discussion

SESSION 4: What Can We Learn from More Detailed Analysis of the Phenotype and the Environment?

Chairperson: P. Sullivan, University of North Carolina, Chapel Hill

N. Freimer, University of California, Los Angeles: Genetics of brain and behavior: What is the right phenotype?

A. McIntosh, University of Edinburgh, United Kingdom: Quantification and stratification of depression for gene discovery.

M. Burmeister, University of Michigan, Ann Arbor: Gene *x* environment interactions play a role in human behavior and psychiatric disorders.

J. Smoller, Harvard Medical School, Boston, Massachusetts: DSM and beyond: Leveraging alternative phenotypic strategies.

General Discussion

SESSION 5: What Can We Learn from Using Alternative Approaches?

Chairperson: W.R. McCombie, Cold Spring Harbor Laboratory

A. Need, Imperial College, London, United Kingdom: Whole-genome sequencing in childhood psychiatric illness.

A. Palotie, University of Helsinki, Finland: Use of population isolates in disease genetics.

SESSION 6: How to Proceed to Understanding More About the Function

Chairperson: N. Freimer, University of California, Los Angeles

K. Brennand, Mount Sinai School of Medicine, New York: Validating genetic findings using human iPSCs.

W.R. McCombie, Cold Spring Harbor Laboratory: Heterogeneity and strategy: Ways to move to function.

Final Discussion: What Next?

Coffee break discussion

Accelerate Genomic Research with Privacy Protections

December 11–13

FUNDED BY **Illumina, Inc.**

ARRANGED BY **Y. Erlich,** Whitehead Institute, Cambridge, Massachusetts
R. Kain, Illumina, Inc., San Diego, California
A. Narayanan, Princeton University, New Jersey

In a decade, we have gone from sequencing megabases of DNA at great cost, to sequencing gigabases at low cost. Projects on a scale unimaginable a few years ago are now possible. The data from these projects, coupled with the healthcare records and other details of the life histories of the individuals, will be the foundation for a revolution in healthcare. One particular challenge is the personal privacy and the ultimate security of personal genome information. Even if the information is used in a de-identified manner for large-scale health studies, there is no guarantee that the information will not be traced back to the individual. There is a danger that the dialogue about the security of an individual's genome information will be driven by anecdotes and ill-considered reporting in scientific journals, the mainstream press, and social networks. We hope to minimize this risk by initiating a public discussion of these issues, recognizing the ethical and technical challenges of managing genomic information and suggesting possible solutions. To do so, we are bringing together scientists from the fields of human genetics, bioinformatics, cryptography, and privacy scholarship.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

What the Meeting Is About: Y. Erlich, Whitehead Institute, Cambridge, Massachusetts

Introduction: Genomics and the Value of Data Dissemination
T. Manolio, National Human Genome Research Institute, Rockville, Maryland

SESSION 1: Anatomy of Hacks: Vulnerability Points and Areas of Vulnerability

Chairperson: R. Kain, Illumina, Inc., San Diego, California

B. Malin, Vanderbilt University, Nashville, Tennessee: Genomics and the wonderful world of re-identification.

Y. Erlich, Whitehead Institute, Cambridge, Massachusetts: Identifying personal genomics by surname inference.

General Discussion

SESSION 2: Current State of Ecosystem: Summary of Ethical and Policy Considerations

Chairperson: R. Kain, Illumina, Inc., San Diego, California

N. Farahany, Duke University, Durham, North Carolina: US and EU privacy frameworks for genomics.

SESSION 3: Approaches to Risk Analysis: Future Vulnerability and Impact

Chairperson: R. Kain, Illumina, Inc., San Diego, California

S. Brenner, University of California, Berkeley: Premises in genome privacy.

D. Glazer, Google, Mountain View, California: Google's privacy principles.

General Discussion

SESSION 4: The Sandbox Model and Access Control

Chairperson: Y. Erlich, Whitehead Institute, Cambridge, Massachusetts

S. Sherry, National Library of Medicine, Bethesda, Maryland: An overview of DBGap, content, format, and access.

R. Shelton, Private Access, Inc., Irvine, California: Consumer-controlled empowered tools for harmonizing privacy and access to confidential information.

A. Philippakis, Broad Institute, Boston, Massachusetts: Genome Bridge, a cloud-based platform for genome-scale analysis: How it works, how is privacy managed.

General Discussion

SESSION 5: Differential Privacy: Quantitative Methods for Data Perturbation or Restricting Queries to Ensure Privacy

Chairperson: Y. Erlich, Whitehead Institute, Cambridge, Massachusetts

A. Narayanan, Princeton University, New Jersey: Introduction and framing: Cryptography and differential privacy (why it's included).

V. Shmatikov, University of Texas, Austin: Attempting to apply differential privacy to genome-wide association studies.

General Discussion

SESSION 6: Cryptographic Approaches for Data Dissemination

Chairperson: Y. Erlich, Whitehead Institute, Cambridge, Massachusetts

E. Eskin, University of California, Los Angeles: Identifying genetic relatives without compromising privacy.

J. Witkowski, S. Turner, M. Olson, T. Hunkapiller

R. Shelton, Y. Erlich, and T. Manolio list topics for discussion

G. Tsudik, University of California, Irvine: How medical predictions can be made from DNA data using homomorphic encryption.

General Discussion

SESSION 7: Interdependencies: Technology Solutions vs. Ethics and Public Policy/Legislation

Chairperson: A. Narayanan, Princeton University, New Jersey

T. Callaghan, Federal Bureau of Investigation, Quantico, Virginia: Law enforcement databases: Tensions between forensic analysis and genetic privacy.

C. Ball, Ancestry DNA, San Francisco, California: DTC testing and the consumer's attitude toward genetic privacy.

L. Rodriguez, National Human Genome Research Institute, Bethesda, Maryland: Moving forward: Evolving policy considerations regarding genomic privacy.

General Discussion

SESSION 8: Summaries and Proposed Outcomes

White paper: Which five key points to include?

Summary and Concluding Remarks

L. Stein, C. Ball

Phelan-McDermid Syndrome: Autism due to Shank3 Mutations/Deletions

December 15–17

FUNDED BY **Phelan-McDermid Syndrome Foundation**

ARRANGED BY **G. Bliss,** Phelan-McDermid Syndrome Foundation, Houston, Texas
R. Dolmetsch, Allen Brain Institute, Stanford, California
C. Powell, University of Texas Southwestern, Dallas

The goals of this discussion meeting were to share the most current research on Shank3-related neurodevelopmental disorders and to design a plan for near-term and long-term research aimed at understanding and treating Shank3-related symptoms. A carefully selected group of scientists and clinicians from diverse backgrounds and interests discussed their most recent data and their candid thoughts on the most promising future avenues of research. Participants shared unpublished data, future research plans, and constructive criticism of published data in the field. Discussion sessions with appointed leaders were interleaved among the talks to encourage goal-directed brainstorming that was hoped would lead to clear future objectives. It was expected that at the conclusion of the meeting, participants would come away with a clear picture of the challenges that lie ahead and strategies to overcome them.

K. Phelan

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Toward a Natural History of PMS

G. Bliss, Phelan-McDermid Syndrome Foundation, Houston, Texas: Registry.
C. Betancur, INSERM-CNRS, Paris, France: Clinical genetics.
K. Phelan, Tulane University School of Medicine, New Orleans, Louisiana: PMS in adolescents and adults.
A. Kolevzon, Icahn School of Medicine at Mt. Sinai, New York: Natural history.

Session Discussion: How to Proceed to Obtaining Information about Natural History?
Moderator: A. Hardan, Stanford University, California

SESSION 2: Toward Clinical Trial Design in PMS

J. Buxbaum and A. Kolevzon, Icahn School of Medicine at Mt. Sinai, New York: PMS clinical trial: Rationale, design, outcome measures.
J. Neul, Baylor College of Medicine, Houston, Texas: Rett syndrome clinical trial outcome measures.
R. Carpenter, Seaside Therapeutics, Cambridge, Massachusetts, and M. Bear, Massachusetts Institute of Technology: Fragile X clinical trial outcome measures.
M. Sahin, Children's Hospital, Boston, Massachusetts: TSC clinical trial outcome measures; Mechanisms of neurocognitive dysfunction and treatment trials in TSC.

Session Discussion: How to Discover and Define Most Appropriate Outcome Measures for Clinical Trials in PMS?
Moderator: J. Veenstra-Vanderweele, Vanderbilt University, Nashville, Tennessee

SESSION 3: Therapeutic Target Identification for PMS in Model Systems: Study, Replicate, Repeat

R. Dolmetsch, Novartis Institute for BioMedical Research, Inc., Cambridge, Massachusetts: Human neuronal cultures.

C. Powell, G. Bliss

R. Dolmetsch

C. Sala, CNR: Institute of Neuroscience, Milan, Italy: Animal neuronal cultures.
Z. Yan, State University of New York, Buffalo: Shank3 deficiency causes synaptic and behavioral impairment via an actin-dependent mechanism.

Session Discussion: In Vitro Models: How Best to Use Them?
Moderator: W. Spooren, F. Hoffmann-LaRoche Ltd., Basel, Switzerland
Do the cultures and animal models overlap?
How can we move rationally to high-throughput screening that is meaningful?
Consensus on how targets should be preclinically validated?

SESSION 3 (continued): Therapeutic Target Identification for PMS in Model Systems

J. Buxbaum, Icahn School of Medicine at Mt. Sinai, New York: Animal models 1: Exon 4–9 (Ankryin repeat domain).
C. Powell, University of Texas, Dallas: Animal models 3: Exon 21 (Homer-binding domain).
Y.-H. Jiang, Duke Institute for Brain Sciences, Durham, North Carolina: Animal models 4: Complete deletion.
J. Holder, Baylor College of Medicine, Houston, Texas: Animal models 5: SHANK3 overexpression; Overexpression of Shank3 causes a unique neuropsychiatric disorder.

Session Discussion: Animal Models: How Best to Use Them?
Moderator: T. Boeckers, Universität Ulm, Germany
What replicates, what does not, why?
What convergence is there if any?
What brain regions are most critical?
What treatments should be studied next in the models?
What are the key issues for future research?

SESSION 4: Goal-Directed Group Discussion

D. Bredt, Johnson & Johnson Pharmaceutical R&D, San Diego, California: Preclinical studies.
W. Kaufmann, Boston Children's Hospital, Massachusetts: Clinical studies and outcome measures.
C. Powell, University of Texas, Dallas: Therapeutic targets and screening.
W. Spooren, F. Hoffmann-LaRoche Ltd., Basel, Switzerland: EU-AIMS.

Final Discussion: What Are the Key Issues, What to Do Next?

- Toward a natural history of PMS
- Toward clinical trial design in PMS
- Therapeutic target identification for PMS in model systems
- Funding
- Dissemination


DNA LEARNING CENTER

DNA LEARNING CENTER
EXECUTIVE DIRECTOR'S REPORT

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The DNALC originated the concept of providing lab experiences in genetics and biotechnology to large numbers of biology students. During the year, we touched base with 13 programs worldwide that were developed under licensing agreements with the DNALC, were initiated by DNALC staff or kick-off events, or that claimed to be directly modeled after us. Together with us, these programs provide hands-on experiments to more than 150,000 students per year. In addition, 25 kits developed with Carolina Biological Supply Company are used by 200,000 students annually. Likely a similar number of students do labs based on our three textbooks and eight experiment websites.

Worldwide Genetics and Biotech Lab Exposures at Programs Modeled on the DNALC

Program	Location	Year founded	Number of labs	Yearly student exposure	Cumulative student exposure
DNA Learning Center, Cold Spring Harbor Laboratory	Cold Spring Harbor, NY	1988	6	31,900	475,580
Biotechnology Teaching Center at Stony Brook University	Stony Brook, NY	1995	3	4,610	75,950
Bay Area Biotechnology Education Consortium (BABEC)	San Francisco Bay, CA	1996	Distributed	45,500	200,000
Ecole de l'ADN ("DNA School"), Museum of Nimes	Nimes, France	1998	9		35,000
Glasemes Labor ("Visible Lab"), GmbH Campus Berlin-Buch	Berlin-Buch, Germany	1999	3	10,045	122,000
Gene Technology Access Centre (GTAC)	Melbourne, Australia	2000	2	11,020	61,430
Life Learning Center, University of Bologna	Bologna, Italy	2000	4	12,910	103,230
Biogen-Idec Community Laboratory	Cambridge, MA	2002	2	2,480	25,430
Lifelab, Life Science Centre	Newcastle upon Tyne, UK	2002	1	3,320	42,430
DNA Learning Lab, Science Centre, Singapore	Singapore	2003	3	24,140	214,270
South Carolina DNA Learning Centre, Clemson University	Clemson, SC	2006	2	1,560	11,740
Vienna Open Lab	Vienna, Austria	2006	1	5,050	29,790
Sackler Educational Lab, American Museum of Natural History	New York, NY	2007	1	3,800	21,330
College of Science, University of Notre Dame	South Bend, IN	2013	1		
			38	**156,335**	**1,418,180**
DNALC Kits, Carolina Biological Supply Company	Burlington, NC	1986		200,000	
				356,335	

In 2008, we collaborated with the New York City (NYC) Department of Education (DOE) to open the *Harlem DNA Lab*, which is located in the John S. Roberts Educational Complex on 1st Avenue and 120th Street, owned by the DOE. During its 5 years of operation, *Harlem DNA Lab* has fulfilled our goal of providing underserved schools in NYC enrichment opportunities identical

to those that we offer Long Island's affluent schools. More than 15,400 students have done hands-on experiments at the facility; 75% were Hispanic or African American. The same proportion of students were from Title 1 schools, at which 40% or more of students are designated as low income. *Harlem DNA Lab* was the site of a Howard Hughes Medical Institute (HHMI) teacher-training program that we administered in collaboration with the NYC DOE. During the 5-year term of the grant, 835 NYC teachers completed an average of 15 hours of training.

The success of *Harlem DNA Lab* has proven that we can find our way in the megalithic NYC school system, draw significant numbers of underrepresented minority (URM) students, and have a measurable impact on student achievement. With no long-term contract with the DOE, use of the *Harlem DNA Lab* continues on a year-to-year basis. Evening, weekend, or holiday access requires permits for each activity. Furthermore, in the last 2 years, there have been several instances of gun violence in the neighborhood that have caused the facility to be "locked down" and have discouraged some schools from attending labs there.

DNA Center NYC

Emboldened by our success, but sobered by the limitations in Harlem, in 2013 we took major steps toward achieving our long-held goal of having our own dedicated center in Manhattan. *DNA Center NYC* will further extend the DNALC "brand" to NYC, using a successful educational formula perfected over 25 years: academic year lab field trips for 5th through 12th grade students, in-school instruction with mobile experiment "footlockers," intensive summer DNA camps, and teacher training and follow-up support for effective hands-on science learning.

DNA Center NYC will be a 7,000–10,000-square-foot facility, administering a full program of student enrichment for 5th–12th grade students from the five boroughs of NYC. Its mission is to be the "go-to" place for DNA education and information from the world's top-rated molecular biology and genetics research institution. *DNA Center NYC* will be a vibrant hub of hands-on explorations of the DNA world and its relationship to inheritance and health. *DNA Center NYC* envisions a day when all NYC students will have the opportunity to look at their own DNA—to better understand their uniqueness, the implications of personalized medicine, and the genetic heritage

Concept illustrations (see also next page) of *DNA Center NYC* provided by Centerbrook Architects and Planners.

they share with other people in America's melting pot. *DNA Center NYC* will leverage the DNALC's leadership to create a unique environment in which students can ask questions and do hands-on science experiments.

Each year, we anticipate providing hands-on labs for 27,500 students and supporting a network of 200 students and mentors involved in extended research projects in DNA barcoding, microbiomes, and genome sequencing. We also plan to train 100 5th- through 12th-grade teachers and provide equipment footlockers to support 2000 in-school student labs. We will continue to encourage participation of URM. To aid recruitment, we will deepen relationships with leaders and programs serving these populations and have budgeted funds to provide scholarships to 50% of all students attending programs at *DNA Center NYC* who are URM and/or from Title I schools.

Because *Harlem DNA Lab* is convenient to students commuting from northern Manhattan and the Bronx, we intend to continue to operate this facility along with the new *DNA Center NYC*. The two facilities will offer the same lab programs, allowing us to serve the broadest possible population. We anticipate that a proportion of *Harlem DNA Lab*'s natural clientele, for example, some schools in the Bronx, will opt to travel to the larger *DNA Center NYC*. However, our 10 years of experience with an analogous situation on Long Island with the Dolan DNALC and DNALC *West* shows that a smaller satellite facility can operate at capacity due to the strong demand for our programs.

Building out and equipping three teaching labs, an exhibit, a bioinformatics/multimedia classroom, a lunchroom, and staff offices will cost $2.7 million. Annual operating costs, including salaries for three administrative staff, six instructional staff, and two interns, are estimated at $2 million. Expected revenues from student labs, interpretive programs, teacher training, and memberships can only generate about half of this amount. Federal and private foundation grants currently supply the difference between operating income and total DNALC operating costs. In light of the decreasing availability of grants and increased funding gaps caused by vagaries of the federal budget process, we have adopted a plan to cover additional operating costs from endowment.

The Cold Spring Harbor Laboratory (CSHL) Board of Trustees has included $25 million for *DNA Center NYC* in its $250 million capital campaign to commemorate the institution's 125th anniversary in 2015. Building on a lead gift of $6 million from CSHL Trustee Laurie Landeau, the campaign gained momentum in the fall. In October, we received a $10 million gift from the Thompson Family Foundation whose namesake, Wade Thompson, founded Thor Industries as one of the world's largest manufacturers of recreational vehicles, including the flagship Airstream. This was followed by a $3 million grant from the Alfred P. Sloan Foundation, prompting CSHL President Bruce Stillman to officially announce the project at the annual Double Helix Award dinner on November 4.

The Sloan Foundation grant provided start-up funds to initiate a three-phase development timetable. By year's end, we had launched Phase I. Working with Cushman & Wakefield Realtors, we began our search for a suitable lease property with good street visibility and ready access to the NYC subway. At the same time, members of a newly formed *DNA Center NYC* Council are introducing the project to companies, foundations, and individuals capable of making major gifts to close out the $25 million capital campaign. We intend to begin Phase II facility development in summer 2014, with design and construction complete in 2015. We have developed a financial plan in which the Sloan grant will fund renovation and staff recruitment in Phases I and II. Then,

income from a $22 million endowment will supplement tuition income in Phase III, producing a sustainable, balanced budget. During Phase III, we will open *DNA Center NYC* and scale up to full operation in 2016.

DNALC Licensing

Beginning with a 2002 agreement with the North Shore–Long Island Jewish Health System that founded DNALC *West,* the licensing program has provided an efficient mechanism for institutions to develop science centers based on the DNALC model. This formal relationship provides transparent access to teaching methods and intellectual property ("know-how") developed over a 25-year period at a cost of more than $40 million.

On September 28, the DNALC at the University of Notre Dame was dedicated, becoming the sixth licensed DNALC operation. The collaboration was made possible by CSHL friend and Notre Dame Alumnus John Passarelli. A 36-seat lab, which makes use of the DNALC's distinctive lab "islands," will be located in the new Jordan Hall of Science. The facility was blessed by a Notre Dame priest—but the football team had no such help when they lost to Oklahoma that afternoon.

By year's end, we also had a draft licensing agreement with Beijing No. 166 High School, a public school with 2000 students in grades 7–12. Beijing No. 166 is the top rated of ~30 high schools in the Dongcheng District in the heart of old Beijing, just one mile from the Forbidden City and Tiananmen Square. Founded in 1864 as China's most prestigious girls' school, Beijing No. 166 became coeducational after the Cultural Revolution. With training in biology, Principal Wang Lei has recently developed Beijing No. 166 as the only designated high school of biology in the capital. The school has major partnerships with the Genetics Society of China and the Beijing Genomics Institute (BGI), whose Chairman, Henry Yang, introduced DNALC Executive Director Dave Micklos to China in 2003.

Beijing No. 166 students pose with DNALC instructional staff following summer workshops.

Our collaboration began in 2011, with 20 students attending a 3-week summer workshop at the DNALC. We continued summer workshops in 2012 and 2013 and added a winter internship for selected students. We were lucky to catch the beginning of the wave of precollege Chinese students visiting the United States, driven by the desire of many top Chinese students to attend U.S. universities and affluent parents who are able to focus resources on a single child. Travel visas have become easier to obtain, and travel agencies are catering to the demand.

The formal collaboration will begin in spring 2014 with funding from the Dongcheng District. Under terms of the agreement, we will create a co-branded DNALC at Beijing No. 166 as a resource for Dongcheng public schools, which serve 100,000 students. A well-equipped biology lab, completed during the renovation of the school in 2013, will be dedicated to providing academic-year field trips and summer camps. The DNALC will provide 260 student-weeks of workshops at Cold Spring Harbor, as well as 4 weeks of student/faculty training in Beijing.

New Program on Next-Generation Sequencing

Since the advent of so-called "next-generation sequencing" (NGS) in 2005, the cost of DNA sequencing has decreased 10,000-fold (imagine anything in your lifetime that has decreased by even twofold!). Coupled with free online analysis tools, NGS offers the promise to make genome

analysis an egalitarian pursuit open to virtually anyone. Indeed, recent DNALC surveys of attendees at scientific meetings and training workshops suggest that 98% of researchers are currently, or soon will be, using large sequence data sets.

Raw DNA and RNA sequences are becoming the currency of modern biology, and easy access to genome information is leading a paradigm shift in the field. Throughout history, biologists have worked lifetimes attempting to amass enough data to support their ideas, with the effect that hypotheses were usually undersupported by data. Today, NGS has created a virtually data-unlimited paradigm in which hypotheses are often derived from sequence data itself. This whole-genome paradigm will dominate the biological landscape for students seeking future careers in biomedical and agricultural research.

Against this backdrop, in the fall, the DNALC was awarded a $537,000 grant from the National Science Foundation (NSF) to develop "Infrastructure and Training to Bring NGS Analysis into Undergraduate Education." This 3-year project will assist undergraduate faculty in integrating NGS analysis into course-based and independent student research. Participating faculty will develop a total of 30 RNA sequence (RNA-Seq) data sets that bear on novel research problems in eukaryotic genomics. Following refinement of a biochemical and bioinformatics workflow by project staff, a Working Group retreat will be conducted at CSHL in June 2014 with 11 faculty. In subsequent years, regional and virtual workshops will be held for 80 faculty representing diverse institutions and areas of the country. Approximately 25% of faculty will be from minority-serving institutions with the objective of reaching African American, Hispanic, and Native American faculty and students.

Analysis will use large-scale data storage and bioinformatics workflows provided by the *iPlant Collaborative*, an NSF-supported cyberinfrastructure for biological research. The project will provide faculty at predominately undergraduate institutions (PUI) the first easy access to high-performance computing through the NSF's Extreme Science and Engineering Discovery Environment (XSEDE). Advanced applications, including command line customization, are supported in the research-grade *Discovery Environment.*

Using the visual metaphor of a subway map, the *DNA Subway* educational platform bundles research-grade bioinformatics tools and databases into intuitive workflows and presents them in an easy-to-use interface. Each of four *DNA Subway* lines focuses on different problems in genome analysis, organizing bioinformatics tools at stops along branches off a main track. The Green Line is an educational workflow specifically designed to support student analysis of RNA-Seq data sets. It integrates all tools needed to assemble millions of RNA sequences into a transcriptome—the entire set of genes active under different conditions or in different cell types (such as normal vs. tumor cells). The Green Line articulates directly with high-performance clusters at the Texas Advanced Computing Center, providing what we believe to be the first easy biological on-ramp to the national supercomputing highway. This infrastructure will make it possible to broadly disseminate on-demand experiments using RNA-Seq in undergraduate settings.

During the grant, training will transition from in-person workshops to online webinars and self-paced learning via a dedicated Internet microsite, providing a sustainable method to introduce large numbers of faculty to NGS analysis. Participants will also share instructional strategies and solve analysis problems during regular webinars and video conferences broadcast from the DNALC's Laurie J. Landeau Multimedia Studio. A multifaceted evaluation program will assess effects on student learning, interests, and attitudes across a variety of classroom and student research settings.

This project operates on the continuum of biology research and education. It recognizes that many college faculty would like to bring NGS to bear on a problem of their own interest and invite students to become coinvestigators of class-based and independent projects. The program will prepare faculty to operate in the new, sequence-driven paradigm and empower them to guide students in novel genome explorations.

iPlant Collaborative

In September, we learned that the NSF had approved an additional 5 years of funding for the *iPlant Collaborative*. The renewal proposal received an extremely rigorous review, including an anonymous panel of peers, a "reverse" site visit with NSF program officials, and approval by NSF division directors, culminating in a final nod from the National Science Board, NSF's governing body. Considering the federal sequester and general uncertainty of research funding, the renewal of this project for $50 million was a vote of confidence in this collaboration among CSHL, the University of Texas, and the University of Arizona.

During the first 5 years of the project, the DNALC successfully expanded its role to become the leader of Education, Outreach, and Training (EOT). This ramped up funding to nearly $800,000 per year, or about one quarter of the DNALC's $3.2 million operating budget. So we heaved a sigh of relief at the renewal. Notably, *iPlant* support allowed the DNALC to recruit two high-level computer programmers, who worked to consolidate our leadership in educational bioinformatics. We focused much effort on *DNA Subway*, finalizing the Green Line in time for the NGS program. With nearly 6000 registered users, *DNA Subway* continues to fulfill our mission of bringing cyberinfrastructure into the classroom, making it easy for students to work with the same data and bioinformatics tools as high-level researchers. The Blue Line articulates with a complete set of lab materials for DNA barcoding and is the basis of the successful *Urban Barcode Project* (UBP), which has involved more than 400 students in examining biodiversity in

(*Top*) The *iPlant Collaborative* website was redesigned during the year and is set to launch in early 2014. Junior designer Chris Weidler created the look of the new site. (*Bottom*) The *Tools & Services* Workshop at CSHL in December was also broadcast using Adobe Connect and Livestream. Watch it here: http://new.livestream.com/cshl-dnalc/cshl-tsw.

NYC. Students analyze and compare DNA barcode sequences and construct phylogenetic trees. An export feature simplifies barcode sequence submissions to GenBank and has been used to publish more than 100 novel DNA sequences, with students as authors.

We are currently upgrading the existing Red Line annotation workflow to incorporate JBrowse and WebApollo, a user-friendly rewrite of the Apollo annotation editor. The Red Line will enable an easy annotation "round trip" as locally generated RNA-Seq data are automatically transferred from the Green Line as annotation evidence. The workflow will readily accept any type of GFF (general feature format) file, including output from Maker and evidence from other genome resources. This seamless integration will create a "power desktop" that allows faculty and students to explore large-scale variation in gene expression and genome structure on their personal computers. We believe that the Green and Red Lines of *DNA Subway* will be the most user-friendly workflows available to support distributed annotation of newly sequenced and re-sequenced genomes. The maize genome is specifically being developed as the first use case.

Also in light of the renewal, we designed a refreshed *iPlant* website that features the variety of *iPlant* platforms and tools and user success stories to highlight the research problems that *iPlant* can help solve. The DNALC will also continue the development of the *iPlant Learning Center*, where users can access *Quick Start* or in-depth tutorials online.

Throughout 2013, we continued to deliver *iPlant* workshops for researchers and faculty. We trained 283 researchers at eight *Tools & Services* Workshops and 38 faculty at *Genomics in Education* Workshops. Workshops and conferences continued to be an important component of reaching and training new *iPlant* users, and thus we anticipated reaching many more people virtually. In 2013, we launched a series of webinars, described further in our *BioMedia* section below. We can achieve an extended reach through webinars; for example, a workshop attended by 27 researchers at CSHL had 316 "virtual" webinar attendees! In total, we reached an additional 405 researchers and faculty virtually.

Longitudinal evaluation continues to guide and inform EOT efforts and document our success in reaching our intermediate audience of biological researchers who do not have specialized expertise in computation. Workshop follow-up surveys show that 60% of *Tools & Services* Workshop attendees actively use the *iPlant* platform to analyze their data, and 72% say that they introduced their colleagues to *iPlant*. *Genomics in Education* Workshops also had significant impact, with faculty reporting more than 24,000 student exposures to *iPlant* bioinformatics exercises as a result.

Genomic Approaches in BioSciences

This year was the final year of our NSF Advanced Technological Education (ATE) grant, which reached 258 biotechnology educators at 12-week-long workshops conducted at community colleges nationwide. The curriculum covers four key technologies: polymerase chain reaction (PCR), DNA sequencing, RNA interference (RNAi), and bioinformatics. In 2013, the last five workshops were held at Tulsa Community College (Tulsa, OK), City College San Francisco (San Francisco, CA), Minneapolis Community and Technical College (Minneapolis, MN), Seminole State College (Sanford, FL), and Nassau Community College (Garden City, NY). Of 103 participants in 2013, 53% were from 2-year colleges, 18% from 4-year colleges or universities, and 28% from high schools, with 30% URM. We conducted follow-up workshops in the spring for 21 educators who attended workshops in 2012.

The workshops consistently increased educator knowledge and confidence to teach labs and bioinformatics. Preworkshop ($n = 260$) and postworkshop ($n = 245$) survey data showed that before the workshop, only 10% of participants knew "a lot" about the key genomic concepts compared with 37% afterward. Participants also felt more confident in teaching the genomics labs (16% to 41%) and bioinformatics (8% to 32%). In long-term follow-up surveys ($n = 98$), 89%

of workshop alumni had implemented course materials at schools with an average of 35% URM students. Educators taught DNA barcoding (52%), PCR (54%), RNAi (25%), bioinformatics (50%), and careers modules (70%), with an annual student exposure of 9768 wet labs, 5582 bioinformatics exercises, and 5304 biotech careers activities.

In January, four case studies were conducted with 2011 workshop alumni. The case studies involved classroom observation of PCR and DNA barcoding ($n = 64$ students, 22% URM), student focus groups ($n = 28$; 25% URM), and educator interviews ($n = 5$) at a rural high school and at suburban 2- and 4-year colleges. College faculty discussed using the curricula as an "enabler" to link subjects, make best use of faculty expertise and equipment, and increase student interest. For example, biotechnology students used DNA barcoding to help ecology students map campus biodiversity. Two courses were implemented as a direct result of our training program. The aim of the ATE program is to "prepare tomorrow's biotechnology workforce," and this was illustrated by one case study participant: *"We've had a lot of interesting classes but Biotech is the only one where we've learned stuff that we can apply when we got a job. . . where I feel a little more confident. . . I could be without supervision."*

Our evaluation data consistently show that bioinformatics is challenging to teach and learn. Both educators and students feel more comfortable and engaged when bioinformatics exercises are incorporated into relevant, personalized curricula, such as our human mitochondrial DNA, DNA barcoding, and RNAi labs. At the least complex end of the spectrum, students use their own mitochondrial DNA sequences to discover principles of human evolution through database searches for similar sequences and alignment to the sequences of other people, primates, and even extinct hominid ancestors. Of medium complexity, DNA barcoding allows students to conduct field work and then use DNA sequences to search databases and build phylogenetic trees to answer questions about species diversity and conservation. In more complex experiments, students use bioinformatics to identify homologs of human disease genes in *Caenorhabditis elegans* and then design targeting vectors to disrupt these genes using RNAi.

In 2013, we secured additional funding to extend the NSF ATE grant and test our "train-the-trainer" model of instruction. Community college cohosts from the original 2011–2013 grant now instruct *Genomic Approaches in BioSciences* Workshops at their colleges. The first "extension" workshop was held at Madison Area Technical College (Madison, Wisconsin), with local faculty teaching the program to 10 participants under DNALC supervision. These workshops will continue in 2014, and we will compare outcomes for extension workshop participants with the original cohort.

Professional Meetings

The DNALC delivered five presentations at the 2013 National Association of Biology Teachers (NABT) conference in Atlanta, Georgia, continuing our partnership with Carolina Biological Supply Company (CBSC) to significantly expand our presence at teacher professional meetings. CBSC has distributed DNALC experiment kits to science teachers since 1986, and this year, we cosponsored a DNA barcoding workshop, based on the CBSC kit, demonstrating all facets from sample collection, DNA isolation and amplification, and gel electrophoresis, through sequencing and bioinformatic analysis. We presented on the power of DNA barcodes and the use of DNA barcoding to drive independent research in the classroom, and we promoted the new DNALC *Genome Science* textbook and laboratory manual for advanced secondary and postsecondary education. The textbook combines approachable narrative with extensively tested lab exercises that illustrate key concepts of genome biology in humans, invertebrates, and plants. Attendees learned about human mitochondrial DNA sequencing, detecting epigenetic DNA methylation in *Arabidopsis thaliana*, and using *DNA Subway*. The presentations emphasized how *Genome Science* provides a historical and conceptual framework to enhance existing courses, start new courses, and support student research projects.

DNA Barcoding

The second year of the *UBP* came to a successful conclusion in June. This year, 53 teams competed: 144 students from 12 public and seven private high schools across NYC, with 24% URM. Teachers and students attended 20 *Open Lab* sessions at the *Harlem DNA Lab* after school and on weekends, equipment footlockers were used by an additional 93 students, and DNALC staff conducted five school visits to help teams with their experiments and phylogenetic analyses on *DNA Subway*. Students collected more than 1000 samples for DNA sequencing, with more than 2500 single sequences provided by GENEWIZ. Importantly, teams discovered 35 novel DNA sequences, which are being published to GenBank with student authors, making the data freely available for use by other researchers.

Forty-one teams presented research posters at the American Museum of Natural History (AMNH) on May 29. The projects mapped NYC wildlife, detected food fraud, determined biodiversity of traded species, identified exotic and/or invasive species, and explored the effects of biodiversity on human health. A jury of 27 experts selected the top six finalist teams (15 students), who gave oral presentations at the AMNH on June 5th. George Amato, Director of the Sackler Institute for Comparative Genomics at AMNH, gave the keynote address: "DNA Barcoding of Endangered Species, From Central African Forests to Restaurants in Manhattan." The grand prize winners were Hostos-Lincoln Academy of Science students Kavita Bhikhi and Hillary Ramirez. Their project, *Using DNA Barcodes to Identify Ant Biodiversity in St. Mary's Park, Bronx, New York*, identified seven ant species and found evidence for population structure within the ant species *Tetramorium caespitum*. Kavita and Hillary built on their research from the 2012 *UBP*, for which they earned fourth prize. This was also mentor Allison Granberry's second *UBP* victory, and her experience is featured in the February 2014 *Carolina Tips*.

(*Top*) Hostos-Lincoln Academy of Science students Hillary Ramirez and Kavita Bhikhi, pictured with Allison Granberry (*center*), won the grand prize. (*Bottom*) *UBP* participants presented research results in a poster session.

Program evaluation highlighted two main barriers to students completing their *UBP* projects: Some teachers were limited in their ability to mentor students through all phases of their research, and students lacked specific training. Therefore, we expanded the program to offer select students a more in-depth experience and direct student-scientist mentoring. We partnered with the Pinkerton Foundation to establish the *Urban Barcode Research Program* (*UBRP*). All *UBRP* students take introductory courses on DNA barcoding, conservation biology or genetics, proposal writing, and laboratory safety, and an expert scientific mentor works directly with each team. *UBRP* students commit 100+ hours to the program, half of which is directed research with their scientist mentor.

In 2013, 126 NYC students completed preparatory *UBRP* workshops at the *Harlem DNA Lab*, AMNH, and the Gateway Institute for Pre-College Science Education at Benjamin N. Cardozo High School in Queens, New York. Students were recruited from DNALC summer camps at *Harlem DNA Lab* in collaboration with the NYC DOE and through the Gateway Institute. At the beginning of the 2013–2014 academic year, 40 of these students were accepted into the first *UBRP* cohort, with 50% URM or disadvantaged students. Teams of two students were paired with scientist mentors from collaborating institutes, such as the Rockefeller University, Columbia University, and the New York Botanical Garden, to guide them through the research process:

Antonia Florio (*top left photo, far left*) leads *DNA Barcoding Research* Workshop students on a specimen collection trip on the CSHL campus.

preparing a proposal, collecting samples, extracting and amplifying DNA, analyzing data, and preparing a scientific poster. As with the *UBP*, projects will culminate in presentations at a research symposium.

Both cycles of the *UBP* and *UBRP* continue into 2014, with a total of 55 student teams participating: 153 students, 20 teachers, and 20 scientists from 33 different NYC high schools and institutions. The students will present their findings at symposia in spring 2014.

To assess the impact of our NYC barcoding research programs, all students complete pre- and postsurveys, and we interview a subcohort of students and teachers. When asked to compare DNA barcoding with other research experiences, *UBP* students felt that the DNA barcoding programs provided more "real world" science (81% of students), more chance for hands-on experience (69%) and to learn science (76%), more opportunity to develop critical thinking (83%) and independent inquiry skills (70%), and more understanding of the scientific process (68%). DNA barcoding increased the students' interest in studying science or pursuing a career in science (83%), while still being more fun than other research experiences (84%).

In line with the primary mission of the DNALC, students consistently cited hands-on lab experiences as the most unique, rewarding, and inspiring aspect of the DNA barcoding programs. As one student commented,

> *It really shows you what everyday scientists actually do. They take an unanswered question, they get their hypothesis and they test it and they come up with an answer that nobody knows. Whereas previous projects that I've done. . .you usually know the expected outcome because so many people have done this experiment over and over again.*
>
> Victoria, 10th grade

We held several DNA barcoding summer camps this year, including basic and advanced research workshops. We expanded DNA barcoding to middle school students with great success: 48 students attended the new *Backyard Barcoding* camp. The students worked in groups to identify plants and fillets of fish. Thirty-nine local and international students completed the advanced high school workshop, *DNA Barcoding Research*. Students explored the biodiversity of the intertidal zone of Cold Spring Harbor and compared DNA isolation methods to identify the efficiency of in-house DNA isolations. Students collected and analyzed more than 150 samples that produced 43 novel sequences to be published to GenBank. Most of the barcoding sequences came from small aquatic invertebrates—species that are hard to classify using traditional taxonomy—highlighting the power of DNA barcoding.

Building on the work of the summer campers, we compared existing and new barcoding methods to identify the most robust, cost-efficient DNA extraction and amplification methods equivalent to commercial kits. We determined silica resin is the best medium, being inexpensive (<$0.30/sample, which is a fraction of the cost of commercial kits) and reproducible with almost any plant, fungus, or animal specimen. For DNA amplification, we found that NEB *Taq* 2× Master Mix (12.5 μL) is comparable to Ready-To-Go PCR Beads at a much lower cost ($0.55/sample vs. $1.60/sample), but it requires access to a stable –20°C freezer. Updated protocols with information on reagents, primers, and amplification methods are available on our barcoding website, www.dnabarcoding101.org.

The DNA barcoding program also extended beyond Cold Spring Harbor in 2013, when we took the curriculum and materials to Africa, Europe, and Asia! In January, Micklos cotaught a DNA barcoding course to 10 students at Godfrey Okoye University in Nigeria, identifying several novel sequences of dragonflies. In March, he presented the curricula at the *Genetics Education for the 21st Century* Workshop in Utrecht, the Netherlands, and then coconvened a meeting on DNA barcoding across European science centers at the Wellcome Trust Sanger Institute, Cambridge, also facilitating a workshop to 100 students while there. During a trip to

Students and faculty of Godfrey Okoye University.

China in November, as part of our ongoing collaboration with Beijing No. 166 High School, he led students in investigating local Beijing biodiversity. In addition, Oscar Pineda-Catalan and Christine Marizzi taught the curriculum to 23 teachers and 80 students in Malaysia and Singapore. The bioinformatics analysis of many of these international samples continues back at the DNALC.

High School Research Programs

Our partnership with Cold Spring Harbor High School (CSHHS) continued, offering the *Molecular and Genomic Biology* "capstone" college-level laboratory course. Co-instructed by DNALC and CSHHS faculty Jaak Raadsepp, students devote two classes per day to the year-long course. The 2012–2013 cohort, our eighth class, focused on hands-on experimentation and independent projects across a range of biological systems. The course included units on eugenics, DNA barcoding, RNAi, human genome analysis, bioremediation, and protein modeling. Highlights were student essays on modern parallels to the eugenics movement of the early 20th century, after students visited CSHL Research Archives to examine historical documents; testing the ability of bacteria to bioremediate various substrates; and three-dimensional printing of cancer proteins with guest lecturer Joan Kiely of Stony Brook University's Biotechnology Teaching Center. The students presented their work during a poster session at the CSHL *Cancer Biology and Therapeutics* meeting on April 24.

We also continued our successful partnership with St. Dominic High School in Oyster Bay, offering a 5-month course for students in *Molecular and Genomic Biology*. The course capitalized on St. Dominic's new science building, made possible through a parishioner's generosity. In collaboration with science teacher Teresa Kuehhas, DNALC staff introduced students to experimental methods for future independent projects: species diversity using DNA barcoding, gene function using RNAi in *C. elegans*, and human variation using PCR.

In the fall, Long Island's first regional Doshi Science, Technology, Engineering and Math (STEM) high school opened. The Doshi STEM program partnered with Brookhaven National

Laboratory and the DNALC to prepare students for the competitive and evolving high-tech science arena. The first 20 9th graders were selected by their school districts on the basis of academic performance, recommendations, and core interest in STEM. The DNALC collaboration provided hands-on, inquiry-based science experiments to enhance student understanding of the scientific method while exploring and solving problems. The Doshi students applied these new skills on their first independent research project, examining biodiversity of Caumsett State Historic Park, Garvies Point Museum and Preserve in Glen Cove, a field by Southampton Hospital, a cranberry bog in Amagansett, and parks in Greenport and Cutchogue. The students collected and analyzed 50 samples using DNA barcoding, which will culminate in a symposium in early 2014.

Bruce Nash (*top left*) meets with Doshi STEM collaborators, and Brooke Roeper (*bottom left*) demonstrates pipetting technique to Doshi students.

Student Programs

This year, 20,962 students attended field trips at our three facilities: DNALC, DNALC *West*, and *Harlem DNA Lab*. We reached an additional 10,200 students through in-school instruction by DNALC staff and 1641 through in-school lab exposures by DNALC-trained teachers with footlocker kits. GENEWIZ processed a total of 17,632 sequencing samples (8030 mitochondrial control region sequences and 8401 DNA barcodes) from students at 194 high schools and 105 colleges and universities.

Of the students who visited the *Harlem DNA Lab,* 70% received scholarships from the William Townsend Porter Foundation. Two schools co-located within the John S. Roberts Educational Complex also received instruction: Coalition School for Social Justice students participated in three high school biotechnology laboratories, and MS 45 students enjoyed six introductory genetics laboratories. Students performed DNA extractions at the *STEM Careers EXPO Fair for English Language Learners* in the Armory on the Hudson and observed mutant fruit flies at the New York Public Library Youth STEM Fair. Through continued collaboration with the NYC DOE Office of School Programs and Partnerships, we held a 5-day *DNA Science* camp during spring break. Twenty-seven budding scientists gave up well-deserved vacations to be immersed in the lab. The highlight of the week was a visit by Dennis Walcott (*right*, *center*), the former Chancellor of the NYC DOE.

The *Harlem DNA Lab* continued its footlocker kit rental program by providing Howard Hughes Medical Institute–trained teachers with equipment and materials for genetics and biotechnology labs. In 2013, footlocker kits resulted in 1566 total student exposures; 74% of these students were URM, and most teachers received the kit free of charge or were charged only minimal restocking fees. In addition, the *Harlem DNA Lab* continued to be the central hub of the *UBP*.

Additional grants from Bank of America and National Grid Foundation supported programs for more than 3000 underserved students from Long Island school districts including the Brentwood, Uniondale, William Floyd, Central Islip, and Valley Stream public schools. As part of an ongoing collaboration with Central Islip Union Free School District (UFSD), we provided in-school labs for every 6th grader as part of the district's new *Exploration Academy Campus*. A customized sequence of labs was presented for both the *Forensic and Legal Sciences* and *Health and Medical Sciences* academies at the Charles Mulligan and Ralph Reed Middle Schools. The program culminated with a visit from Bob Keller, Executive Director of the National Grid Foundation, and Craig Carr, Superintendent of the Central Islip UFSD, to celebrate their accomplishments in the lab.

We continued to work with Charter Member schools. The Chapin School introduced an advanced science elective, *Molecular Genetics*. The course curriculum, based on DNALC's *Genome Science* textbook, is implemented with the assistance of DNALC instructors. The Convent of the Sacred Heart School in Greenwich, Connecticut, continued field trips to the DNALC, in-school instruction, and summer camps, and also expanded its research programs. St. David's School in Manhattan joined this year with a focus on enriching the 5th-grade science curriculum through field trips and in-school instruction for a genetics unit that culminates in a DNA barcoding project and a school science fair.

This summer, 1241 students participated in 60 week-long camps held at nine locations: DNALC, DNALC *West*, Stony Brook University, and Brookhaven National Laboratory (Long Island); *Harlem DNA Lab*, the Chapin School, and New World Prep Charter School (NYC); Convent of the Sacred Heart (CT); and the Roxbury Latin School (MA).

The summer roster was enhanced by the addition of our new *BioCoding* summer camp. The DNALC has a long interest in bringing bioinformatics education to students. Since 1998, students have used our websites such as *BioServers* to analyze their own mitochondrial DNA sequence, simulate genetic drift, and investigate other areas of computational biology. With cheap DNA sequencing now a reality, bioinformatics skills—the ability to manipulate software and computational resources to unlock the meaning of sequence—are now the bottleneck to discovery. This course was pioneered by DNALC programmers Sheldon McKay and Mohammed Khalfan, along with Jason Williams. Although the world of bioinformatics is intimidating (even to many biologists today), 82% of the students who attended had little or no coding experience. Our summer camp increased 91% of students' interest in coding, with 73% very likely to now take a science or computer science elective at school. We are confident our camp prepares students for an academic world where, to quote one famous CSHL bioinformatician, Lincoln Stein, "if you can't do bioinformatics, you can't do biology."

In 2013, the DNALC helped to establish the i2 ("iNVENT and iNSPIRE") summer camps in NYC and Boston. Founded by Ethan Berman, the objective of the i2 camps is to broaden students' exposure to STEM with a wide variety of new, hands-on, and innovative courses not seen in traditional middle school science education. As part of i2, the Roxbury Latin School in Massachusetts and the Chapin School in Manhattan offered three DNALC middle school programs: *Fun with DNA*, *World of Enzymes*, and *Forensic Detectives.* Each course was taught by a DNALC lead teacher, an associate teacher, and a high school student acting as a "near peer." Our programs reached 73 students and feedback was overwhelmingly positive. i2 plans to continue this collaboration by offering DNALC summer camps in additional national and international locations next summer.

Each year, we collaborate with the North Shore–Long Island Jewish (NS-LIJ) health system to give students the opportunity to step into biotechnology teachers' shoes. The I.S. 059 Springfield Gardens School in Jamaica, Queens, sends select 8th- through 11th-grade students to DNALC *West*, located in the NS-LIJ core laboratory at Lake Success, to learn the latest biotechnology and bioinformatics techniques. Those "seed students" then teach their

classmates back at I.S. 059. Since 2008, 125 students have been through the program, with 16 attending in 2013. "We had tons of fun—learned, taught. Thanks for giving us gifts that will keep on giving" (Marcia Young, I.S. 059 science teacher). The larger aim of the program is to allow less-fortunate students an opportunity to experience the laboratory environment and gain exposure to a variety of career choices, and we are tracking program alumni regarding STEM career choices.

Monthly *Saturday DNA!* sessions drew 221 participants, with parents and grandparents joining children to learn about forensics, cholesterol, human origins, genetic traits, the Romanov family mystery, genes for bioluminescence, and DNA barcoding. An additional 3426 visitors viewed films in the multitorium, including Cablevision's multimedia presentation *Long Island Discovery*, and/or viewed the exhibition *The Genes We Share*. The annual *Great Moments in DNA Science* seminar series drew 126 top Long Island high school students for three presentations by CSHL researchers. Dr. Jaclyn Novatt showed how basic research on nuclear pores is improving drug production. Dr. Aman Husbands talked about his research on plant leaf architecture and cell differentiation; and Dr. Dennis Eckmeier discussed how mice rely on scent during social interactions.

Renovations to our exhibit space continued as we updated "Our Common Origins" to showcase the latest fossil finds, including *Ardipithecus ramidus* or "Ardi," which is more than 4 million years old. We redesigned the skull wall and displayed a phylogenetic tree behind the skulls, describing genetic and morphological relationships between species leading to modern man. More than 100 classes per year take an instructor-guided tour of our museum, so we updated the accompanying booklet that helps them on their journey and that teachers use to extend field trips back in the classroom.

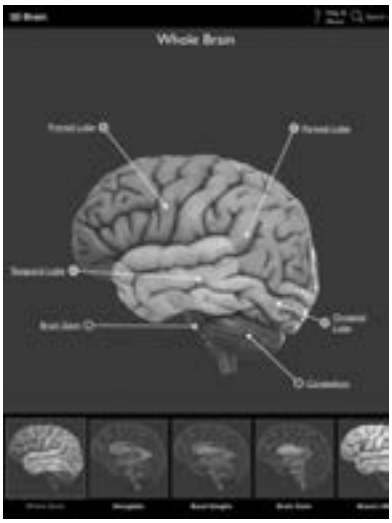
Graduate Training

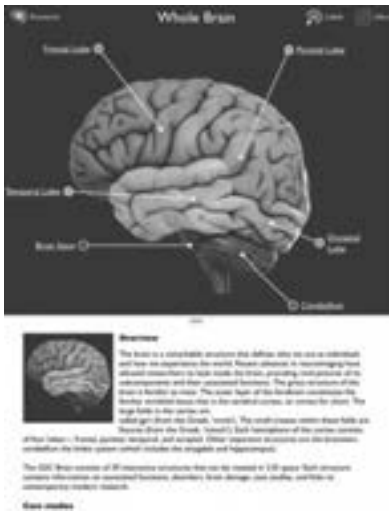
Through our collaboration with the Watson School of Biological Sciences, graduate students develop presentation methods that can be used with any audience. The spring rotation includes teaching both middle school and high school students at the DNALC. While under the guidance of experienced DNALC instructors, graduate students work in pairs to complete 12 half-day teaching sessions, learning how to assess an audience quickly and build quality instruction accordingly.

We continue to collaborate with the New York Academy of Sciences (NYAS), one of the oldest scientific institutions in the United States and a leading organizer of scientific meetings. DNALC instructors trained 56 mentors in effective strategies to deliver hands-on genetics activities and forensics training in after-school settings for the NYAS science mentoring program.

BioMedia *Visitation and Projects*

We had a record number of visitors to our suite of multimedia resources in 2013. *Google Analytics* counted 4,861,670 visits to 22 DNALC websites. Our *YouTube* videos received 823,400 views,





and the *3D Brain*, *Weed to Wonder*, and *Gene Screen* apps were downloaded 579,163 times. Therefore, total website, *YouTube*, and smartphone/tablet apps visitation was 6.26 million, an 11% increase over 2012.

If you blinked or went to the kitchen, you may have missed it, but the DNALC's *3D Brain* (see photos above) app was featured in Apple ads that aired during the Academy Awards. Developed under a grant from the Dana Foundation, the iPhone, iPad, and iPod app has been downloaded 1.6 million times and is one of the most highly rated education apps on iTunes.

Since its launch in 2009, *3D Brain* has proven to be one of the most successful resources we have ever produced, with 2.2 million downloads to date on Apple, Windows, and Android devices worldwide! Reviews show that the app is used primarily by teachers and students, as intended, but also by health professionals, patients, and the public: *As a psych student, this info is constantly brought up in lectures and tutorials—it's great [I can use] this without Internet access! Great on-hand app!* and *This is a fantastic app, possibly the best medical/anatomy app I have seen. As a nurse, it has been very helpful for me when I work with patients with acquired brain injuries.*

In October, we released an update for the Apple version of the *3D Brain* with current information for all 29 brain structures. The update was installed on 485,000 devices, showing that the *3D Brain* was still being used by 29% of people who had downloaded it to an Apple device (1,652,638). We took the opportunity to offer users an "in-app" purchase of *3D Brain HQ,* which includes higher-resolution images and a redesigned user interface for $0.99. Despite an increasing percentage of free apps in app stores, at year's end, we tallied a respectable 11,322 in-app purchases of the *HQ* version. Proceeds from in-app purchases support the educational programs of the DNALC.

In 2013, we moved into live streaming media and webinars to more fully use the DNALC's Laurie J. Landeau Multimedia Studio. We upgraded our capabilities with an all-in-one portable live production switcher, called the Livestream HD 500, which is built to television standards. This portable PC CPU/monitor enables real-time switching among multiple cameras (e.g., between a wide shot and close-ups of laboratory bench techniques), the presenter's computer screen, a video clip, and graphics overlay titles. We can simultaneously stream and record high-definition video, whether in the studio or on the road. The streaming video is distributed to end users via a DNALC Adobe Connect "meeting room" for events with less than 50 participants or the DNALC

webpage on the Livestream server for larger events. Adobe Connect features a customizable meeting space for document downloads and live chats. The recorded events are then posted to the relevant DNALC websites, Livestream page, and YouTube channel.

Our first live event was the *UBP* Award Ceremony on June 5th. The event included a keynote address by George Amato, Director of the Sackler Institute of Comparative Genomics; student presentations; and awards. On August 9th, we broadcasted *"DNA for Short": The Story of a Most Important Discovery, A Conversation with Dr. James D. Watson*. Dr. Watson met with middle school students attending a *Fun with DNA* summer camp at the DNALC and recounted the story of the discovery of the structure of DNA. Campers' parents and invited guests watched from the DNALC Multitorium, and 175 people viewed the event on Livestream.

In the fall, we presented a series of 11 webinars. The inaugural webinar was follow-up to a high school human mitochondrial DNA laboratory taught by Dave Micklos in the Faroe Islands, off the northwest coast of Denmark. Dave reminded 41 students of the theory behind the lab and then walked them through their results and analyses using the DNALC's *BioServers* and *DNA Subway* websites. The next series of webinars were targeted at *iPlant* users and educators doing DNA barcoding and included introductory tutorials as well as refresher courses in wet labs and bioinformatics, with 88 participants, many of them repeat attendees. In 2014, we will expand our webinars to include "virtual" follow-ups for field trips and summer camps.

(*Top*) James Watson and *Fun with DNA* campers pose following the *DNA for Short* live broadcast. (*Bottom*) Jermel Watkins demonstrates the updated barcoding silica DNA isolation technique during an *iPlant* webinar. Chun-hua Yang (*far right*) is manning the close-up camera, and the Livestream HD 500 switcher is shown at left.

To promote our expanding international programs, we also produced three videos in Chinese, summarizing the history of CSHL and the DNALC and our mission to be a pioneer in genetics education, providing case studies of students from Beijing No. 166 High School attending summer workshops.

Staff and Interns

There were many staff changes in 2013. We are pleased to say that our staff family has grown and evolved, but we are also sad to see some of our colleagues move on to other opportunities.

Dr. Oscar Pineda-Catalan joined the DNALC in 2011 as *UBP* Manager, founding the competition in 2012. He also developed and taught innovative barcoding programs for both teachers and high school students at the DNALC and offsite. Oscar moved on in the spring, becoming

2013 new DNALC employees Antonia Florio (*left*) and Catherine Zhang.

Manager of the Science Research Mentoring Program (SRMP) at the AMNH.

Oscar recommended his AMNH colleague Dr. Antonia Florio as his replacement, and she filled the role of Conservation Genetics instructor in March. Growing up, Antonia had two mantras: "Animals are cool, and being outside is fun," which spawned her love for biology and the natural world. She received her undergraduate degree at Macauley Honors College (CUNY City College), doing research in the Galapagos, French Guiana, and South Africa. She then completed her doctorate in comparative biology of Madagascan chameleon species at the AMNH. Antonia both manages and provides instruction for NYC-based DNA barcoding projects (the *UBP* and *UBRP*) and assists with lab instruction at all three DNALC teaching facilities.

Cornel Ghiban, Computer Programmer, left in the spring to join Estée Lauder as a Perl programmer. Even before he was hired in 2006 and came to the United States from Romania, he did contract work for us, developing the "back end" of websites. While at the DNALC, he developed and maintained the expanding suite of existing DNALC websites and applications and worked on *DNA Subway*. Cornel continues a working relationship with us on development of *DNA Subway*.

We also bid farewell to computer programmer Sheldon McKay, who joined us in 2011, moving from *iPlant* in Tucson, Arizona. He developed and taught advanced-level bioinformatics and *iPlant* cyberinfrastructure and managed outreach and educational activities. Sheldon is now with former CSHL scientist Lincoln Stein at the Ontario Institute for Cancer Research, working with the Reactome database for metabolic pathways and biochemical reactions.

A new position was created for Dr. Xiaoquin "Catherine" Zhang as Manager of International Collaborations. After receiving her medical degree at Xian Medical University, China, in 1995, she received a Master of Science from Johns Hopkins University in Baltimore, Maryland, majoring in biotechnology and bioinformatics. Before joining our team, Catherine was an oncology research associate in CSHL Professor Nicholas Tonks' lab and helped to organize the first two Beijing No. 166 High School summer camps in 2011 and 2012. Catherine develops collaborations with several groups in China and is also a high school instructor.

In the fall, Tony Biondo, our Junior Programmer, left to go to law school at St. Johns University, New York. Tony assisted with the back end of many *BioMedia* websites and continues to help us occasionally with projects. Also this year, one of our longest-serving interns, Christian Weidler, was promoted to Junior Designer in the *BioMedia* Group after receiving his Bachelor of Technology in Visual Communications from Farmingdale State College.

Since opening in 1988, we have successfully integrated high school and college interns into every aspect of our day-to-day operations. During the course of a paid internship, students progress from preparing reagents for middle school labs to preparing DNA samples for sequencing and maintaining a worldwide catalog of orders for RNAi projects. In addition to prep work, interns may take on independent or team projects mentored by DNALC staff members, which translate current research into classroom labs. The *BioMedia* department also welcomes interns for summer or longer-term roles. An internship offers students unique opportunities to gain real laboratory or design experience in an educational environment.

Interns also conduct external research, such as the study of the environmental management of *Alliaria petiolata*, an invasive species of garlic mustard, conducted by Robert Scott (Sage College). Robert placed third in the New York State *Science & Engineering Intel Competition*. Brittany Coscio (St. Anthony's High School, Huntington, New York) completed research on the effect of multitasking on students' performance. Arielle Bryan (City College of New York) also completed an honors thesis analyzing the *Drosophila melanogaster* cactus protein via allele sequencing.

We gathered an amazing group of interns this year and said farewell as others left for college.

New Interns

*Joseph Babinski, Northeastern University, Chemical Engineering**
Kayla Bianco, St. Anthony's High School
Gabriella Blazich, Our Lady of Mercy High School
*Kalliopi Chatzis, New York Institute of Technology, Doctor of Osteopathy**
Brittany Coscio, St. Anthony's High School
*Paul Donat, SUNY Geneseo, Biochemistry**
Kenny Escobar,* Freeport High School*
Nella Hauser, Stony Brook University, Biology
*Julie Hemphill, Penn State University, Neuroscience**
Ashleigh Jackobel, Farmingdale State College, Biosciences
Jungseo (Joe) Kim, Locust Valley High School*
Michaela Lee, Syosset High School
William Manolarakis, Chaminade High School*
Jack Manzi, Kings Park High School
Anant Mehrotra, Oyster Bay High School
Giovanni Sanchez, Jericho High School*
*Shenika Shah, Fordham University, Natural Sciences**
Katherine Villalon, John Jay College of Criminal Justice, Forensics
*Isabell Whiteley, *Boston University, Neuroscience*

Departing for College

Matt Angeliadis, University of Connecticut, Molecular and Cell Biology
Katie Belissimo, College of New Jersey, Biology
Anne Bode, Notre Dame University, Biological Sciences
Arielle Bryan, City College of New York, Biology
David Dopfel, Stony Brook University, Biomedical Engineering
Magdalene Economou, Georgetown University, Biology
Pauline McGlone, University of Delaware, Medical Diagnostics
Sophie Podhurst, Northeastern University, Chemical Engineering
Daliah Ross, New York University, Neuroscience
Robert Scott, Sage College of Albany, Clinical Biology
Sulaiman Usman, New York Institute of Technology, Life Sciences
Lina Marie Varghese, Stony Brook University, Biology

* Summer only. *College students in italics.*

2013 Workshops, Meetings, Collaborations, and Site Visits

January 4	Feinstein Institute, "DNA Learning Center" Presentation, Manhasset, New York
January 4–5	Godfrey Okoye University, "DNA Barcoding and *DNA Subway*" Workshop, Enugu, Nigeria
January 7–8	Lone Star College CyFair, "DNA Barcoding" Workshop, Kingwood, Texas
January 11	International Plant and Animal Genome XXI Conference 2013, pre-PAG Half-Day, NSF *iPlant Collaborative Tools & Services* Workshop, Town and Country Convention Center, San Diego, California
January 12	*Saturday DNA!* "Bad Cholesterol"! DNALC
January 12	STEM Mentor Training, New York Academy of Sciences, New York, New York
January 19	The New York Academy of Sciences, *Fun with DNA* and *Enzyme Labs for Middle School Instruction* Workshops, Albany, New York
January 21	"DNA Learning Center" and "DNA Barcoding" Presentations, Singapore Science Center, Jurong East
Jan. 23–Feb. 1	"Nobelist Mindset Program" Workshop, National University of Malaysia, Kuala Lumpur
Jan. 25–Feb. 6	Visiting Student Internships, Beijing No. 166 High School, Beijing, China, DNALC
January 29	Site visit by John Ettinger and Betsy Fader, Helmsley Trust, New York
February 1	Nassau County Science Supervisors Meeting, "DNALC Education Programs" Presentation, Nassau County BOCES, Garden City, New York
February 2	*Saturday DNA!* "CSI Chapters: The Science of Blood Spatter," DNALC
February 2	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
February 7	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
February 13	*Urban Barcode Project* Open Lab, Brooklyn Technical High School, New York
February 14	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
February 16	National Biotechnology Education Conference, *DNA Barcoding and DNA Subway* Workshop, Santa Clara University, California
February 19	Site visit by Sir William and Renice Castell, Wellcome Trust Sanger Institute, Hinxton, Cambridge, England
February 21	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
February 23	*Human Mitochondrial Sequencing* Workshop, Center for Work Education, City University of New York, New York
February 26	Site visit by Jeanne Garbarino, The Rockefeller University, New York
March 2	NSF ATE Professional Development Genomic Approaches in Biosciences Follow-up Workshop, Austin Community College, Texas
March 2	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 4–12	Visiting Student Internship, Godfrey Okoye University, Enugu, Nigeria, DNALC
March 5	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*

March 7	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 9	*Saturday DNA!* "Exploring Human Origins –What DNA Says About Our Ancestry," DNALC
March 9	*Saturday DNA!* Third Grade Program, CSHL
March 9	*Urban Barcode Project* Open Lab, Trinity School, New York
March 9	*Urban Barcode Project* Open Lab, The Chapin School, New York
March 12	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 14	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 14–17	NSF *iPlant Collaborative Tools & Services* Workshop, Maize Genetics Conference, St. Charles, Illinois
March 19	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 21	*Great Moments in DNA Science* Honors Seminar, "Learning from Nature: Making Filters by Copying Nature's Nuclear Pore Complex," Jaclyn Novatt, CSHL, DNALC
March 21	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
March 21–22	Wellcome Trust Sanger Institute, *DNA Barcoding and DNA Subway* Workshop, Hinxton, Cambridge, England
March 23	New York Public Library Youth STEM Fair, "Fruit Flies" Presentation Booth, Schomburg Center for Research in Black Culture, New York
March 25–29	*DNA Science* Workshop, *Harlem DNA Lab*
March 29	Site visit by Dennis Walcott, New York City Department of Education, *Harlem DNA Lab*
April 4	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 6	North Carolina Academy of Science, University of North Carolina, "Engineering Society: The American Eugenics Movement" Seminar, Pembroke, North Carolina
April 6	North Carolina Academy of Science, University of North Carolina, "*iPlant Collaborative* Project" Seminar, Pembroke, North Carolina
April 6	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 8	National Association for Research in Science Teaching, "Bioinformatics Tools and Databases in the Biology Classroom" Seminar, Rio Grande, Puerto Rico
April 8–19	Visiting Student Internship, Godfrey Okoye University, Enugu, Nigeria, DNALC
April 9	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 10	Site visit by Noah Heller, Math for America, New York, *Harlem DNA Lab*
April 11	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 16	Site visit by Lydia Begley, Nassau County BOCES, Garden City, New York
April 16	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 18	*Great Moments in DNA Science* Honors Seminar, "The Curious Case of Developmental Biology, or Why Do Organisms Always Become What They Are Supposed to?" Aman Husbands, CSHL, DNALC
April 18	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 19	"DNA Extraction" Workshop, STEM Careers EXPO Fair for English Language Learners, Armory on the Hudson, New York
April 20	*Saturday DNA!* "Mendel, Mendel, How Does Your Garden Grow?" DNALC
April 20	Stony Brook Science and Arts EXPO, "Banana DNA Extraction," Stony Brook University, Stony Brook, New York
April 23	Pinkerton *DNA Barcoding* Course, American Museum of Natural History, New York
April 24	Site visit by Keith W. Jones, Brookhaven National Laboratory, Upton, New York
April 25	Pinkerton *DNA Barcoding* Course, American Museum of Natural History, New York
April 25	"DNA Detectives" Course, Lehman College, New York
April 26	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
April 27	NSF ATE *Genomic Approaches in BioSciences* Follow-up Workshop, Bluegrass Community and Technical College, Lexington, Kentucky
April 27	NSF ATE, *Genomic Approaches in BioSciences* Follow-up Workshop, Southern Maine Community College, South Portland
April 30	*Great Moments in DNA Science* Honors Seminar, "Learning to Smell Each Other?," Dennis Eckmeier, CSHL, DNALC
April 30	Pinkerton *DNA Barcoding* Course, American Museum of Natural History, New York
April 30	*DNA Detectives* Course, Lehman College, New York
May 3	*Urban Barcode Project* Open Lab, *Harlem DNA Lab*
May 6	Site visit by Sherry Zhang, Medical College of Wisconsin, Milwaukee, Wisconsin
May 9	Site visit by Tatiana Nikolenko, Rusnano, Moscow, Russia
May 10	Site visit by Theodore Muth, Brooklyn College, New York
May 11	*Saturday DNA!* "DNA: Innocent or Guilty?," DNALC
May 13	Site visit by Marino Golinelli, Life Learning Center and Fondazione Marino Golinelli, Bologna, Italy
May 13–14	Site visit by Delegation from University of Notre Dame, Indiana
May 13–17	NSF ATE *Genomic Approaches in BioSciences* Workshop, Tulsa Community College, Oklahoma

May 16	Pinkerton *DNA Barcoding* Course, American Museum of Natural History, New York
May 16	"DNA Detectives" Course, Lehman College, New York
May 21	Pinkerton *DNA Barcoding* Course, American Museum of Natural History, New York
May 21	*DNA Detectives* Course, Lehman College, New York
May 23	Pinkerton *DNA Barcoding* Course, American Museum of Natural History, New York
May 23	*DNA Detectives* Course, Lehman College, New York
May 28	Pinkerton *DNA Barcoding* Course, American Museum of Natural History, New York
May 28	*DNA Detectives* Course, Lehman College, New York
May 29	*Urban Barcode Project* Poster Session, American Museum of Natural History, New York
May 30	Site visit by Alan Siegel, Thompson Family Foundation, Dallas, Texas
May 30	*DNA Detectives* Course, Lehman College, New York
June 2–3	World Science Festival Presentation, "Pocket Service: Gene Screen and 3-D Brain," New York
June 4	Bio-Link Summer Fellows Forum, University of California, Berkeley, "DNA Barcoding in the Classroom"
June 4	Site visit by Dr. Christian Anieke, Dr. Christian Okeke, Dr. Obiora Ike, Godfrey Okoye University, Enugu, Nigeria
June 4	"DNA Detectives" Course, Lehman College, New York
June 5	*Urban Barcode Project* Final Symposium and Awards Ceremony, American Museum of Natural History, New York
June 6	"DNA Detectives" Course, Lehman College, New York
June 8	*Saturday DNA!* "The Mystery of Anastasia," DNALC
June 10	Site visit by David Heller, Allan Morrison, Phil Ferralli, and Beth Paine, Carolina Biological Supply Company, Burlington, North Carolina
June 10–14	*Fun with DNA* Workshop, Convent of the Sacred Heart, Greenwich, Connecticut
	Forensics Workshop, Convent of the Sacred Heart, Greenwich, Connecticut
June 11	Site visit by Allan Dobrin, Gillian Small, Iris Weinshall, and Alexandra Logue, City University of New York, New York
June 11	20th Annual Golf Outing, Piping Rock Club, Locust Valley, New York
June 17–21	*Fun with DNA* Workshop, Roxbury Latin School, Boston, Massachusetts
June 17–21	NSF ATE *Genomic Approaches in Biosciences* Supplemental Workshop, Madison Area Technical College, Wisconsin
June 19–25	*Fun with DNA* Workshop, The Chapin School, New York
June 21	Site visit by Harriett Copel, Long Island Matrix of Science and Technology, Upton, New York; Ken White, Brookhaven National Laboratory, Upton, New York; Tom Rogers and Lydia Begley, Nassau BOCES; and Scott and Leena Doshi, Doshi Family Foundation
June 24–28	*World of Enzymes* Workshop, Roxbury Latin School, Boston, Massachusetts
June 24–28	Pinkerton *Conservation Genetics* Workshop, *Harlem DNA Lab*
June 24–28	*DNA Science* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Green Genes Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Fun with DNA Workshop, DNA Learning Center *West*
June 26–July 2	*World of Enzymes* Workshop, The Chapin School, New York
July 1–5	*DNA Barcoding Research* Workshop, DNALC
	Forensic Detectives Workshop, DNALC
	World of Enzymes Workshop, DNALC
	World of Enzymes Workshop, DNA Learning Center *West*
July 8–12	NSF ATE *Genomic Approaches in BioSciences* Workshop, City College of San Francisco, California
July 8–12	*Forensics* Workshop, Roxbury Latin School, Boston, Massachusetts
July 8–12	*Forensics* Workshop, The Chapin School, New York
July 8–12	*DNA Barcoding Research* Workshop, DNALC
	DNA Science Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNA Learning Center *West*
July 9	Advanced Placement Biology Institute, Rice University, "Human Evolution and Population Genetics" Workshop, Houston, Texas
July 15–19	*BioCoding* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Silencing Genomes Workshop, DNALC
	World of Enzymes Workshop, DNALC

July 16	Advanced Placement Biology Institute, "Human Evolution and Population Genetics" Workshop, West Lake High School, Denver, Colorado
	Green Genes Workshop, DNA Learning Center *West*
July 20–24	American Association of Plant Biologists, Plant Biology 2013, "*iPlant Collaborative*: A Unified Cyberinfrastructure for a Unified Research Paradigm" Seminar, Providence, Rhode Island
July 22–26	Pinkerton *DNA Barcoding* Workshop, *Harlem DNA Lab*
July 22–26	*DNA Barcoding* Workshop, The Rockefeller University, New York
July 22–26	*DNA Science* Workshop, DNALC
	Forensic Detectives Workshop, DNALC
	Green Genes Workshop, DNALC
	Fun with DNA Workshop, DNA Learning Center *West*
	Fun with DNA Workshop, New World Preparatory School, Staten Island, New York
July 28	Botanical Society of America, Botany 2013, *Genomics in Education* Workshop and NSF *iPlant Collaborative Tools & Services* Workshop, New Orleans, Louisiana
July 29–Aug. 2	*Barcoding* Workshop, DNALC
	Backyard Barcoding Workshop, DNALC
	Fun with DNA Workshop, DNALC
	World of Enzymes, DNALC
	DNA Science Workshop, DNA Learning Center *West*
August 5–9	Pinkerton *Conservation Genetics* and *DNA Barcoding* Workshop, *Harlem DNA Lab*
August 5–9	*DNA Science* Workshop, DNALC
	Forensic Detectives Workshop, DNALC
	Fun with DNA Workshop, DNALC
	DNA Science Workshop, Stony Brook University, Stony Brook, New York
August 5–9	NSF ATE *Genomic Approaches in BioSciences* Workshop, Minneapolis Community and Technical College, Minnesota
August 12–16	*Backyard Barcoding* Workshop, DNALC
	DNA Science Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Genome Science Workshop, DNA Learning Center *West*
	DNA Science Workshop, *Harlem DNA Lab*
	Fun with DNA Workshop, Brookhaven National Laboratory, Upton, New York
August 12–16	NSF ATE *Genomic Approaches in Biosciences* Workshop, Seminole State College, Sanford, Florida
August 16	Pinkerton *Urban Barcode Project Grant Writing* Workshop, *Harlem DNA Lab*
August 19–23	*Fun with DNA* Workshop, DNALC
	Genome Science Workshop, DNALC
	Green Genes Workshop, DNALC
	Silencing Genomes Workshop, DNALC
	World of Enzymes Workshop, DNA Learning Center *West*
	World of Enzymes Workshop, Brookhaven National Laboratory, Upton, New York
August 19–23	NSF ATE *Genomic Approaches in Biosciences* Workshop, Nassau Community College, Garden City, New York
August 26–30	*Backyard Barcoding* Workshop, DNALC
	DNA Science Workshop, DNALC
	Green Genes Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Forensic Detectives Workshop, DNA Learning Center *West*
August 26	Pinkerton *Urban Barcode Project Grant Writing* Workshop, *Harlem DNA Lab*
August 27	National University of Malaysia Delegation, "DNA Barcoding: DNA Isolation and PCR" Workshop, DNALC
August 30	National University of Malaysia Delegation, "DNA Barcoding and Bioinformatics" Workshop, New York Academy of Sciences, New York
Sept. 17–20	NSF *iPlant Collaborative* Professional Development *Tools and Services* Workshop, "Data Mining with *iPlant*," University of Warwick, Coventry, England
September 18	FarGen Summit, "Human DNA Polymorphisms" Workshop, Torshavn, Faroe Islands, Denmark
September 20	FarGen Summit, "Coming Into the Genome Age: The FarGen Exploration," Torshavn, Faroe Islands, Denmark
Sept. 23–24	NSF *iPlant Collaborative Tools & Services* Workshop, Texas A&M University, College Station
Sept. 26–27	NSF *iPlant Collaborative Genomics in Education* Workshop, Prairie View Texas A&M University
October 1	FarGen Summit "Human DNA Polymorphisms" Follow-up Webinar, DNALC
October 3	NSF *iPlant Collaborative Tools & Services* Workshop, New York Botanical Garden, Bronx, New York
October 3–4	Site visit by Stanley Xu and Lin Xu, Taurus Education, Shanghai, China

October 11	NSF *iPlant Collaborative* Webinar co-taught with Carol Lushbough from University of South Dakota, "RNA-Seq & BioExtract," DNALC
Oct. 16–18	NSF *iPlant Collaborative* "Reconnecting with *iPlant*" Webinar Series, DNALC
October 19	*Saturday DNA!* "Glowing Genes," DNALC
October 19	Ant Collection Workshop, Pleasantville Community Garden, East Harlem, New York
October 21	Site visit by Bruno Strasser, University of Geneva, Switzerland
October 22	Site visit by Vicky Han, China Service Center for Educational Exchange, Beijing, China
Oct. 23–25	NSF *iPlant Collaborative* ,"Introduction to *iPlant*" Webinar Series DNALC
Oct. 28–29	NSF *iPlant Collaborative* Professional Development *Tools & Services* Workshop, Colorado State University, Fort Collins
Oct. 28–30	NSF *iPlant Collaborative* "DNA Barcoding for Educators" Webinar Series, DNALC
October 29	Site visit by Sean B. Carroll, Howard Hughes Medical Institute, Chevy Chase, Maryland
November 2	*Urban Barcode Project* Teacher Training, *Harlem DNA Lab*
November 4–5	Site visit by Brent Buckner, Truman State University, Kirksville, Missouri
November 8	Site visit by Regeneron Pharmaceuticals, Tarrytown, New York and Laura Woznitski, BioMed Realty, San Diego, California
November 9	*Saturday DNA!* "Botanical Barcoding," DNALC
November 12	"What DNA Says About Our Human Family" Seminar, Beijing No. 166 High School, China
November 12–14	"Human DNA Polymorphisms, Evolution, and Population Genetics" Workshop, Beijing No. 166 High School, China
November 20–23	National Association of Biology Teachers 2013 Professional Development Conference: "Detecting Epigenetic DNA Methylation in *Arabidopsis thaliana*," "What's in My Sushi? Unlocking the Power of DNA Barcoding," "*DNA Subway*: Cutting-edge Bioinformatics for the Classroom," "*Genome Science:* Biology in the Post-Genome Age," "DNA Barcoding: Independent Research in the Classroom," Atlanta, Georgia
December 4	NSF *iPlant Collaborative Tools and Services* Workshop, Cold Spring Harbor Laboratory
December 4	NSF *iPlant Collaborative* Seminar, "A Unified Cyberinfrastructure for Plant Science," Cold Spring Harbor Laboratory
December 7	*Saturday DNA!* "Dust Away Crime: The Truth about Fingerprints," DNALC
December 18	Site visit by Noah Fuller, New York University, New York

Sites of Major Faculty Workshops

Program Key: *Middle School* High School **College**

State	*Institution*	*Year*
ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	**University of Alaska, Anchorage**	**2012**
	University of Alaska, Fairbanks	1996
ARIZONA	Arizona State University, Tempe	2009
	Tuba City High School	1988
	University of Arizona, Tucson	**2011**
	United States Department of Agriculture, Maricopa	**2012**
ARKANSAS	Henderson State University, Arkadelphia	1992
	University of Arkansas, Little Rock	**2012**
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, 2013**
	Contra Costa County Office of Education, Pleasant Hill	2002, 2009
	Foothill College, Los Altos Hills	**1997**
	Harbor-UCLA Research & Education Institute, Torrance	2003
	Los Angeles Biomedical Research Institute (LA Biomed), Torrance	**2006**
	Laney College, Oakland	1999
	Lutheran University, Thousand Oaks	1999
	Oxnard Community College, Oxnard	2009
	Pasadena City College	**2010**

	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001, 2008
	San Francisco State University	1991
	San Diego State University	**2012**
	San Jose State University	**2005**
	Santa Clara University	2010
	Stanford University, Palo Alto	**2012**
	University of California, Berkeley	**2010, 2012**
	University of California, Davis	1986
	University of California, Davis	**2012**
	University of California, Northridge	**1993**
	University of California, Riverside	2011
	University of California, Riverside	**2012**
COLORADO	Aspen Science Center	2006
	Colorado College, Colorado Springs	1994, 2007
	Colorado State University, Fort Collins	**2013**
	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
	Seminole State College, Sanford	**2013**
	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, 2012**
	Morehouse College, Atlanta	**1991, 1996–1997**
	Spelman College, Atlanta	**2010**
HAWAII	Kamehameha Secondary School, Honolulu	1990
	University of Hawaii at Manoa	**2012**
ILLINOIS	Argonne National Laboratory	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
	Purdue University, West Lafayette	**2012**
IDAHO	University of Idaho, Moscow	1994
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	**Bluegrass Community & Technical College, Lexington**	**2012–2013**
	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Bossier Parish Community College	2009
	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
	Southern University at New Orleans	**2012**
MAINE	Bates College, Lewiston	1995
	Southern Maine Community College	**2012–2013**
	Foundation for Blood Research, Scarborough	2002
MARYLAND	Annapolis Senior High School	1989
	Bowie State University	**2011**
	Frederick Cancer Research Center	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	National Center for Biotechnology Information, Bethesda	2002
	St. John's College, Annapolis	*1991*

	University of Maryland, School of Medicine, Baltimore	**1999**
MASSACHUSETTS	**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	1988
	The Winsor School, Boston	1987
	Whitehead Institute for Biomedical Research, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
	Schoolcraft College, Livonia	**2012**
MINNESOTA	Minneapolis Community and Technical College, Madison	2009
	Minneapolis Community and Technical College, Madison	**2013**
	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
	University of Missouri, Columbia	**2012**
	Washington University, St. Louis	**1989, 1997, 2011**
MONTANA	**Montana State University, Bozeman**	**2012**
NEVADA	University of Nevada, Reno	1992
NEW HAMPSHIRE	Great Bay Community College, Portsmouth	2009
	New Hampshire Community Technical College, Portsmouth	**1999**
	St. Paul's School, Concord	1986–1987
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
	Raritan Valley Community College, Somerville	2009
NEW MEXICO	Biolink Southwest Regional Meeting, Albuquerque	2008
NEW YORK	Albany High School	1987
	American Museum of Natural History, New York	2007
	Bronx High School of Science	1987
	Canisius College, Buffalo	2007
	Canisius College, Buffalo	**2011**
	City College of New York	2012
	Cold Spring Harbor High School	1985, 1987
	Columbia University, New York	**1993**
	Cornell University, Ithaca	**2005**
	DeWitt Middle School, Ithaca	*1991, 1993*
	Dolan DNA Learning Center	1988–1995, 2001–2004, 2006–2009
	Dolan DNA Learning Center	**1990, 1992, 1995, 2000–2011**
	Dolan DNA Learning Center	*1990–1992*
	DNA Learning Center *West*	2005
	Fostertown School, Newburgh	*1991*
	Harlem DNA Lab, East Harlem	2008–2009, 2011–2013
	Huntington High School	1986
	Irvington High School	1986
	John Jay College of Criminal Justice	2009
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mount Sinai School of Medicine, New York	1997
	Nassau Community College, Garden City	**2013**
	New York Botanical Garden, Bronx	**2013**
	New York City Department of Education	2007, 2012
	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
	North Carolina State University, Raleigh	**2012**
NORTH DAKOTA	**North Dakota State University, Fargo**	**2012**
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, Tulsa	2009
	Tulsa Community College, Tulsa	**2012–2013**
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–Roundrock Campus	**2007–2009, 2013**
	Austin Community College–Roundrock Campus	**2012**
	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 University, College Station, TX	**2013**
	Texas A&M University, Prairie View, TX	**2013**
	Texas A&M, AG Research and Extension Center, Weslaco	2007
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999, 2004, 2010, 2012**
	University of Texas, Brownsville	**2010**
UTAH	**Brigham Young University, Provo**	**2012**
	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	2011, 2012
	University of Washington, Seattle	**1993, 1998, 2010**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003
	Madison Area Technical College	**1999, 2009, 2011–2013**
	Marquette University, Milwaukee	1986–1987
	University of Wisconsin, Madison	1988–1989
	University of Wisconsin, Madison	**2004, 2012**
WYOMING	University of Wyoming, Laramie	1991
PUERTO RICO	**Universidad del Turabo, Gurabo, Puerto Rico**	**2011, 2012**
	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

AFRICA	Godfrye Okoye University, Enugu, Nigeria	2013
AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
AUSTRIA	**Vienna Open Lab, Vienna**	**2007, 2012**
CANADA	Red River Community College, Winnipeg, Manitoba	1989
CHINA	Beijing No. 166 High School, Beijing	2013
	Ho Yu College, Hong Kong	**2009**
DENMARK	**Faroe Genome Project, Torshavn, Faroe Islands**	**2013**
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
	Singapore Science Center	2013
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University	2000
THE NETHERLANDS	**International Chromosome Conference, Amsterdam**	**2007**
UNITED KINGDOM	**Wellcome Trust Conference Center, Hinxton**	**2012–2013**
	University of Warwick, Coventry	**2013**

COLD SPRING HARBOR
LABORATORY PRESS

2013 PRESS PUBLICATIONS

Serials

Genes & Development, Vol. 27 (www.genesdev.org)

Genome Research, Vol. 23 (www.genome.org)

Learning & Memory, Vol. 20 (www.learnmem.org)

RNA, Vol. 19 (www.rnajournal.org)

Cold Spring Harbor Symposia in Quantitative Biology, Vol. 77: *The Biology of Plants*, edited by Terri Grodzicker, Robert Martienssen, David Stewart, 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

Antibodies: A Laboratory Manual, Second Edition, edited by Edward A. Greenfield

Purifying and Culturing Neural Cells: A Laboratory Manual, edited by Ben A. Barres and Beth Stevens

Manipulating the Mouse Embryo: A Laboratory Manual, Fourth Edition, by Richard Behringer, Marina Gertsenstein, Kristina Vintersten Nagy, and Andras Nagy

Calcium Techniques: A Laboratory Manual, edited by Jan B. Parys, Martin D. Bootman, David I. Yule, and Geert Bultynck

Mouse Models of Cancer: A Laboratory Manual, edited by Cory Abate-Shen, Katerina Politi, Lewis A. Chodosh, and Kenneth P. Olive

Textbooks

Introduction to Protein–DNA Interactions: Structure, Thermodynamics, and Bioinformatics, by Gary D. Stormo

Molecular Biology of the Gene, Seventh Edition, by James D. Watson, Tania A. Baker, Stephen P. Bell, Alexander Gann, Michael Levine, and Richard Losick

Mammalian Development: Networks, Switches, and Morphogenetic Processes, edited by Patrick P.L. Tam and W. James Nelson

Monographs (Topic Collections from *Perspectives in Biology* and *Perspectives in Medicine*)

The Endoplasmic Reticulum, edited by Susan Ferro-Novick, Tom A. Rapoport, and Randy Schekman

DNA Replication, edited by Stephen D. Bell, Marcel Méchali, and Melvin L. DePamphilis

Immune Tolerance, edited by Diane J. Mathis and Alexander Y. Rudensky

Cystic Fibrosis: A Trilogy of Biochemistry, Physiology, and Therapy, edited by John R. Riordan, Richard C. Boucher, and Paul M. Quinton

Hemoglobin and Its Diseases, edited by David J. Weatherall, Alan N. Schechter, and David G. Nathan

Cell Survival and Cell Death, edited by Eric H. Baehrecke, Douglas R. Green, Sally Kornbluth, and Guy S. Salvesen

DNA Repair, Mutagenesis, and Other Responses to DNA Damage, edited by Errol C. Friedberg, Stephen J. Elledge, Alan R. Lehmann, Tomas Lindahl, and Marco Muzi-Falconi

Mitochondria, edited by Douglas C. Wallace and Richard J. Youle

Signaling by Receptor Tyrosine Kinases, edited by Joseph Schlessinger and Mark A. Lemmon

Transplantation, edited by Laurence A. Turka and Katheryn J. Wood

Bacterial Pathogenesis, edited by Pascale Cossart and Stanley Maloy

History

The Dawn of Human Genetics, by V.V. Babkov, edited by James Schwartz, translated from the Russian by Victor Fet

The Eighth Day of Creation: The Makers of the Revolution in Biology, Commemorative Edition, by Horace Freeland Judson

Blue Skies and Bench Space: Adventures in Cancer Research, by Kathleen Weston

Other

Lab Math: A Handbook of Measurements, Calculations, and Other Quantitative Skills for Use at the Bench, Second Edition, by Dany Adams Spencer

CSHL Annual Report 2012, Yearbook Edition

Banbury Center Annual Report 2012

E-books (Kindle editions)

Next-Generation DNA Sequencing Informatics, by Stuart M. Brown

The Eighth Day of Creation: The Makers of the Revolution in Biology, Commemorative Edition, by Horace Freeland Judson

Websites

Cold Spring Harbor Monographs Archive Online (www.cshmonographs.org)

Cold Spring Harbor Symposium on Quantitative Biology Archive (symposium.cshlp.org)

Blues Skies and Bench Space, author's blog and free download of the book in PDF format (www.blueskiesbenchspace.org)

Next-Generation DNA Sequencing, resources (www.seqinformatics.com)

Services

bioRxiv, the preprint server for biology (www.bioRxiv.org)

BioSupplyNet, scientific supply directory (www.biosupplynet.com)

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

Scientists around the world hold the publications of Cold Spring Harbor Laboratory Press in high esteem. From its modest beginnings in 1933 with a single volume, the Press has established a program consisting of seven journals, 190 books, and online services for research preprints and laboratory products. During the last two decades of extraordinary change in media technologies and business models, the Press has continually adapted and become now a fully digital publisher with expertise and agility in developing and delivering a variety of publication types. Recent changes in Press staffing, skills, and organizational structure have enabled this transformation and created a foundation for continued growth.

Financially, the past year was one of significant challenges for all publishers, including those focused on molecular and cellular biology. In the United States and Europe, budgetary restrictions continued to squeeze research departments and the academic libraries that are the most important purchasers of scientific information. The Press weathered these difficulties by maintaining the first-class editorial standards that are its hallmark while finding fresh ways of promoting its publications to scientists and their institutions worldwide. Aided by the success of three recently launched review journals, and the growth of subscriptions in emerging scientific communities abroad, the Press was able once again to make a significant contribution to the Laboratory's economy.

Widely cited impact-factor measurements rank two Press journals, *Genes & Development* and *Genome Research*, in the top three of the world's genetics journals and in the top 1% overall of the 8000 journals in the *Science Citation Index*. Online usage of these and all the other Press journals continues to climb, exceeding 12 million full-text article downloads in 2013, an increase of 25% year-on-year. *Cold Spring Harbor Protocols* and *Learning & Memory* continued to gain subscriptions and usage. *Cold Spring Harbor Perspectives in Biology* saw particularly sharp growth—a vote of approval for its novel publishing model that integrates journal and book publishing. A testament to editorial quality was the award of Nobel Prizes in 2013 to three of the scientists who edited recent *Perspectives* subject collections (Tom Südhof, Randy Schekman, and Jim Rothman).

The Press published 24 new book titles in 2013. Several were revised editions of past best sellers including *Antibodies: A Laboratory Manual* and *Lab Math: A Handbook of Measurements, Calculations, and Other Quantitative Skills for Use at the Bench*. Eleven books were derived from the two online *Perspectives* review journals.

In November, bioRxiv, a new preprint service for the life sciences, was launched to enable distribution of scientific manuscripts not yet published in peer-reviewed journals. This service enables scientists to rapidly share the results of their work and to benefit from the critique of readers as manuscripts are honed for publication in a peer-reviewed journal. bioRxiv is the latest example of innovation at the Press in its long history of assisting biomedical research and offers another new platform on which to build the next generation of valuable services for scientists.

Staff

During the year, we said farewell to Robert Majovski and marked with sadness the retirement of three staff members who had given invaluable service to the Laboratory in many different capacities: Elizabeth Powers after 19 years, Susan Schaefer after 31 years, and Judy Cuddihy after 35 years. We also welcomed Laura DeMare to the editorial staff of *Genome Research,* Jacqueline

Beggins to Production, and Robert Redmond to Marketing and Sales. A full list of Press staff members as of December 31, 2013, is listed elsewhere in this volume.

As an educational division of the Laboratory, the mission of the Press is to create publications and services that help scientists succeed. To fulfill the mission, we are able to call on many of the world's most accomplished scientists, who respect the Laboratory's uniqueness and drive for excellence in all of its activities. But ultimately, our program relies on the talents of our publishing team. We are fortunate to have skilled and dedicated individuals in all the operations of the Press. I am particularly grateful to those who have leadership roles in our ever more diverse activities: Jan Argentine, Terri Grodzicker, Wayne Manos, Stephen Nussbaum, Richard Sever, Marcie Siconolfi, Hillary Sussman, Linda Sussman, and Denise Weiss. And also to my perpetually patient and cheerful Executive Assistant, Mala Shwe Mazzullo, who does so much to oil the wheels of the organization.

John Inglis
Executive Director
and Publisher

Press Executive Director John Inglis (fourth from right) during a visit with delegates of China's National Library of Science and Technology, Beijing

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2013
(with comparative financial information as of December 31, 2012)

		2013	2012
Assets:			
Cash and cash equivalents	$	65,258,594	75,471,404
Grants receivable		8,506,168	10,012,401
Contributions receivable, net		110,387,860	145,289,626
Publications inventory		2,187,157	2,767,427
Investments		384,070,487	296,611,329
Restricted use assets		4,504,767	3,597,846
Other assets		16,226,007	15,891,942
Land, buildings, and equipment, net		231,988,575	240,625,332
Total assets	$	823,129,615	790,267,307
Liabilities and net assets:			
Liabilities:			
Accounts payable and accrued expenses	$	10,437,314	10,456,412
Deferred revenue		5,209,891	5,258,525
Interest rate swap		18,613,481	35,556,347
Bonds payable		97,200,000	97,200,000
Total liabilities		131,460,686	148,471,284
Net assets:			
Unrestricted		294,223,173	243,281,390
Temporarily restricted		290,273,304	293,464,158
Permanently restricted		107,172,452	105,050,475
Total net assets		691,668,929	641,796,023
Total liabilities and net assets	$	823,129,615	790,267,307

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2013
(with summarized financial information for the year ended December 31, 2012)

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2013 Total*	*2012 Total*
Revenue and other support:					
Public support - contributions and nonfederal grant awards	$ 19,110,366	23,863,425	2,121,977	45,095,768	60,846,197
Federal grant awards	26,766,266	–	–	26,766,266	28,711,510
Indirect cost allowances	23,228,697	–	–	23,228,697	24,445,726
Investment return utilized	14,837,880	–	–	14,837,880	23,396,825
Program fees	7,752,249	–	–	7,752,249	8,049,856
Publications sales	10,174,394	–	–	10,174,394	10,957,863
Dining services	4,715,566	–	–	4,715,566	4,614,691
Rooms and apartments	3,859,785	–	–	3,859,785	3,770,473
Miscellaneous	2,579,049	–	–	2,579,049	2,527,870
Net assets released from restrictions	50,653,554	(50,653,554)	–	–	–
Total revenue and other support	163,677,806	(26,790,129)	2,121,977	139,009,654	167,321,011
Expenses:					
Research	85,776,617	–	–	85,776,617	88,821,283
Educational programs	17,388,667	–	–	17,388,667	17,815,388
Publications	9,412,728	–	–	9,412,728	10,092,372
Banbury Center conferences	1,571,105	–	–	1,571,105	1,389,048
DNA Learning Center programs	1,623,885	–	–	1,623,885	1,578,211
Watson School of Biological Sciences programs	3,370,964	–	–	3,370,964	3,356,033
General and administrative	18,110,232	–	–	18,110,232	16,860,891
Dining services	5,591,701	–	–	5,591,701	5,642,788
Total expenses	142,845,899	–	–	142,845,899	145,556,014
Excess (deficiency) of revenue and other support over (under) expenses	20,831,907	(26,790,129)	2,121,977	(3,836,245)	21,764,997
Other changes in net assets:					
Investment return excluding amount utilized	13,167,010	23,599,275	–	36,766,285	9,639,048
Change in fair value of interest rate swap	16,942,866	–	–	16,942,866	2,170,350
Increase (decrease) in net assets	50,941,783	(3,190,854)	2,121,977	49,872,906	33,574,395
Net assets at beginning of year	243,281,390	293,464,158	105,050,475	641,796,023	608,221,628
Net assets at end of year	$ 294,223,173	290,273,304	107,172,452	691,668,929	641,796,023

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2013
(with comparative financial information for the year ended December 31, 2012)

		2013	2012
Cash flows from operating activities:			
Increase in net assets	$	49,872,906	33,574,395
Adjustments to reconcile increase in net assets to net cash provided by (used in) operating activities:			
Change in fair value of interest rate swap		(16,942,866)	(2,170,350)
Depreciation and amortization		14,816,411	15,422,891
Net appreciation in fair value of investments		(47,898,692)	(29,325,772)
Contributions restricted for long-term investment		(5,308,027)	(10,527,878)
Changes in assets and liabilities:			
Grants receivable		1,506,233	(4,279,297)
Contributions receivable, net of financing activities		16,062,486	25,176,987
Publications inventory		580,270	525,471
Other assets		(283,358)	(912,934)
Restricted use assets		(906,921)	(715,256)
Accounts payable and accrued expenses, net of financing activities		268,487	(139,990)
Deferred revenue		(48,634)	(209,041)
Net cash provided by operating activities		11,718,295	26,419,226
Cash flows from investing activities:			
Capital expenditures		(6,179,654)	(14,219,427)
Proceeds from sales and maturities of investments		119,019,919	72,539,685
Purchases of investments		(158,580,385)	(70,038,915)
Net change in investment in employee residences		(50,707)	48,779
Net cash used in investing activities		(45,790,827)	(11,669,878)
Cash flows from financing activities:			
Contributions restricted for long-term investment		2,121,977	1,147,593
Contributions restricted for investment in capital		3,186,050	9,380,285
Decrease (increase) in contributions receivable		18,839,280	(7,321,968)
Decrease in accounts payable relating to capital expenditures		(287,585)	(539,182)
Net cash provided by financing activities		23,859,722	2,666,728
Net (decrease) increase in cash and cash equivalents		(10,212,810)	17,416,076
Cash and cash equivalents at beginning of year		75,471,404	58,055,329
Cash and cash equivalents at end of year	$	65,258,594	75,471,404
Supplemental disclosure:			
Interest paid	$	4,016,854	3,683,436
Noncash investing and financing activity:			
Contributed property	$	641,995	50,000

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the Dolan DNA Learning Center receive a substantial portion of their funding through grants from the federal government and through grants, capital gifts, and annual contributions from New York State, private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2013.

GRANTS January 1–December 31, 2013

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2013 Funding*[1]	
FEDERAL GRANTS					
NATIONAL INSTITUTES OF HEALTH					
Program Project and Center Support	Drs. Hannon/Krainer/Spector/Stillman	05/25/12	12/31/16	$4,101,060.00	
	Dr. Stillman-Cancer Center Core	08/17/11	07/31/16	4,077,038.00	
Cooperative Research Agreement Support[2]	Dr. Gingeras	09/21/12	07/31/16	1,884,527.00	
	Drs. Lowe/Hannon/Hicks/Powers	09/01/09	08/31/14	523,424.00	
	Drs. Powers/Hannon/Krasnitz/Sordella	05/01/12	04/30/17	971,815.00	
Research Support	Dr. Albeanu	07/01/13	06/30/18	472,500.00	*
	Dr. Churchland	03/01/13	02/28/18	428,000.00	*
	Dr. Dubnau	09/15/09	08/31/14	453,742.00	
	Drs. Enikolopov/Koulakov	09/15/11	05/31/16	379,277.00	
	Dr. Furukawa	03/01/10	02/28/15	449,064.00	
	Dr. Hannon	09/01/13	08/31/18	406,350.00	*
	Dr. Hannon	04/01/13	03/31/15	243,206.00	*
	Dr. Huang	07/01/11	03/31/16	542,447.00	
	Dr. Huang	07/01/13	06/30/18	866,231.00	*
	Drs. Kepecs/Huang	07/01/11	05/31/16	398,968.00	
	Drs. Koulakov/Enikolopov	07/15/10	02/28/14	342,047.00	
	Drs. Koulakov/Zador	07/01/13	03/31/18	425,250.00	*
	Dr. Krainer	04/01/12	03/31/17	708,654.00	
	Dr. Krainer	09/01/12	08/31/14	273,578.00	
	Dr. Li	07/01/10	03/31/15	541,939.00	
	Dr. Martienssen	09/15/11	08/31/15	333,972.00	
	Dr. Martienssen	01/20/12	11/30/15	388,223.00	
	Dr. McCombie	07/23/10	02/28/15	344,653.00	
	Dr. McCombie	08/16/13	06/30/16	733,183.00	*
	Dr. Mitra	09/30/09	03/31/14	813,515.00	
	Dr. Mitra	03/15/13	01/31/16	249,999.00	*
	Dr. Muthuswamy	02/01/09	12/31/14	385,656.00	
	Dr. Osten	04/01/12	03/31/17	448,320.00	
	Dr. D. Spector	04/01/11	03/31/15	720,164.00	
	Dr. Stillman	06/01/12	05/31/16	676,199.00	
	Dr. Tonks	07/01/10	04/30/15	721,714.00	
	Dr. Tonks	01/11/10	12/31/14	423,922.00	
	Dr. Trotman	04/01/10	01/31/14	286,069.00	
	Dr. Turner	07/15/10	06/30/15	434,511.00	

[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 2013

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2013 Funding*[1]	
	Dr. Vakoc	04/01/13	03/31/18	$ 549,047.00	*
	Dr. VanAelst	08/01/13	05/31/18	582,341.00	*
	Dr. VanAelst	01/01/09	12/31/14	357,586.00	
	Dr. VanAelst	04/01/13	03/31/18	481,818.00	*
	Dr. Zador	09/27/10	05/31/15	453,742.00	
	Dr. Zador	09/01/12	08/31/17	381,544.00	
	Dr. Zador	04/01/13	03/31/15	236,250.00	*
Research Subcontracts					
NIH/Certerra, Inc. Consortium Agreement	Dr. Koulakov	01/03/13	12/31/13	84,867.00	*
NIH/Cornell University Consortium Agreement	Dr. Enikolopov	03/01/13	02/29/16	354,936.00	*
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	07/31/15	200,000.00	
NIH/Johns Hopkins Consortium Agreement	Dr. Schatz	09/21/11	08/31/14	98,986.00	
NIH/Scripps Research Institute Consortium Agreement	Dr. Osten	09/30/12	08/31/17	73,332.00	
NIH/Sloan-Kettering Institute for Cancer Research Consortium Agreement	Dr. Sordella	09/12/12	08/31/17	293,452.00	
NIH/University of California–A San Francisco Consortium Agreement	Dr. Egeblad	09/01/09	08/31/14	297,562.00	
NIH/University of Minnesota Consortium Agreement	Dr. Tuveson	07/01/12	06/30/15	190,418.00	
		07/01/10	06/30/14	100,313.00	
NIH/University of Texas Consortium Agreement	Dr. Furukawa	09/30/11	05/31/15	18,700.00	
Fellowship/Career Development Support	Dr. Anczukow	09/01/13	08/31/15	107,088.00	*
	Dr. Jansen	01/01/11	10/15/13	12,276.96	
	S. Kelly	07/16/13	07/15/16	26,232.00	*
	J. Tucciarone	09/17/12	09/16/15	26,232.00	
Institutional Training Program Support	Dr. Gann/Watson School of Biological Sciences	07/01/12	06/30/17	267,927.00	
	Dr. Mills	09/01/11	08/31/16	185,045.00	
Course Support	Advanced Immunocytochemistry: In Situ Hybridization and Live-Cell Imaging	09/01/10	08/31/15	88,154.00	
	Advanced Sequencing Technologies and Applications	04/10/12	03/31/15	66,069.00	
	Cell and Developmental Biology of *Xenopus*	04/01/09	03/31/14	61,428.00	
	Cellular Biology of Addiction	03/01/11	02/29/16	78,505.00	
	Eukaryotic Gene Expression	04/01/12	03/31/17	103,691.96	
	Mouse Development, Stem Cells, and Cancer	04/01/12	03/31/17	119,516.32	
	Neurobiology of *Drosophila*	07/15/12	06/30/17	35,000.00	
	Programming for Biology	09/01/09	08/31/14	63,654.00	
	Protein Purification and Characterization	04/01/12	03/31/17	96,704.72	
	Proteomics	08/01/12	04/30/17	107,840.00	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2013

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2013 Funding*[1]	
	Statistical Methods for Functional Genomics	08/05/13	07/31/17	$ 105,657.00	*
	X-Ray Methods in Structural Biology	09/01/12	08/31/17	81,540.00	
Meeting Support	Behavior and Neurogenetics of Nonhuman Primates	07/01/13	06/30/14	20,000.00	*
	The Biology of Genomes	04/01/13	03/31/14	54,600.00	*
	Cancer Biology and Therapeutics	04/15/13	03/31/14	5,000.00	*
	Cell Death	09/01/13	08/31/14	29,958.00	*
	Eukaryotic DNA Replication	08/01/13	07/31/14	5,000.00	*
	Eukaryotic mRNA Processing	08/01/13	07/31/14	4,000.00	*
	Gene Expression and Signaling in the Immune System	12/15/13	11/30/14	8,000.00	*
	Genome Informatics	09/19/13	08/31/14	31,600.00	*
	Glia Health and Disease	09/30/13	08/31/14	15,000.00	*
	Harnessing Immunity to Prevent and Treat Disease	11/12/13	10/31/14	8,000.00	*
	Metabolic Signaling and Disease: From Cell to Organism	05/22/13	04/30/14	12,000.00	*
	Microbial Pathogenesis and Host Response	09/01/13	08/31/14	7,000.00	*
	Network Biology	04/21/09	02/28/14	5,000.00	
	Neurobiology of *Drosophila*	09/01/13	08/31/14	10,000.00	*
	Neuronal Circuits	09/30/13	09/29/14	20,000.00	*
	Pharmacogenomics and Personalized Therapy	09/30/10	08/31/14	10,000.00	
	Retroviruses	03/15/12	02/28/17	36,645.00	
	RNA and Oligonucleotide Therapeutics	04/01/13	03/31/14	10,000.00	*
	78th Symposium on Quantitative Biology: Immunity and Tolerance	04/01/13	03/31/14	5,000.00	*
	Synapses: From Molecules to Circuits and Behavior	04/01/11	01/31/16	14,400.00	
	Wiring the Brain	07/17/13	07/16/14	10,000.00	*
	Yeast Cell Biology	06/01/11	05/31/16	5,000.00	
NATIONAL SCIENCE FOUNDATION					
Multiple Project Award Support	Dr. Jackson	10/01/10	09/30/15	1,140,498.00	
	Drs. Lippman/Schatz	11/01/12	10/31/15	902,602.00	
	Dr. Mitra	10/01/13	09/30/16	354,881.00	*
	Dr. Ware	06/01/12	05/31/17	2,350,569.00	
Research Support	Dr. Churchland	08/01/11	07/31/14	43,156.00	
	Drs. Timmermans/Hammell	05/01/12	04/30/16	305,429.00	
Research Subcontracts					
NSF/Cornell University Consortium Agreement	Dr. Timmermans	02/01/13	01/31/18	402,885.00	*
	Dr. Ware	05/15/13	04/30/18	144,165.00	*
NSF/Iowa State University Consortium Agreement	Dr. Jackson	03/01/13	02/28/18	379,668.00	*
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	08/01/10	07/31/14	419,685.00	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2013

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NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	03/01/11	02/29/16	$ 451,932.00	
NSF/University of Arizona Consortium Agreement	Dr. Ware	08/15/10	07/31/14	597,678.00	
	Drs. Ware/Micklos/Schatz	09/01/13	08/31/18	1,890,979.00	*
	Drs. Ware/Micklos/Schatz	02/01/12	07/31/13	46,684.06	
Fellowship Support	Dr. Carlston	06/01/13	05/31/14	44,000.00	*
Undergraduate Training Program Support	Dr. Schatz	04/01/12	03/31/15	116,932.00	
Course Support	Advanced Bacterial Genetics	07/01/09	06/30/14	98,081.00	
	Computational Cell Biology	08/01/12	07/31/15	70,000.00	
	Frontiers and Techniques in Plant Science	05/15/12	04/30/15	100,615.00	
	Drosophila Neurobiology: Genes, Circuits, and Behavior	08/01/12	07/31/14	20,000.00	
Meeting Support	Eukaryotic mRNA Processing	07/01/13	06/30/14	5,000.00	*
	Computational Cell Biology	04/15/13	03/31/14	14,660.00	*
UNITED STATES DEPARTMENT OF AGRICULTURE					
Research Support	Dr. Jackson	01/15/11	01/14/15	125,802.00	
	Dr. Jackson	12/01/13	11/30/17	118,556.00	*
	Dr. McCombie	09/11/09	09/10/14	192,673.00	
UNITED STATES DEPARTMENT OF THE ARMY					
Research Support	Dr. Hannon	09/15/12	09/14/17	1,072,791.00	
	Drs. Hicks/Trotman	09/30/12	09/29/14	308,554.84	
Fellowship Support	Dr. Sheppard	09/01/12	08/31/15	54,927.00	
UNITED STATES DEPARTMENT OF ENERGY					
Research Subcontracts					
DOE/Brookhaven National Laboratory Consortium Agreement	Drs. Ware/Schatz	11/29/11	09/30/16	1,050,000.00	
UNITED STATES DEPARTMENT OF THE NAVY					
Course Support	Synthetic Biology	07/01/13	06/30/14	45,552.00	*
MISCELLANEOUS SOURCES OF FUNDING					
Equipment Support					
New York State Department of Economic Development	Dr. Martienssen	11/01/12	10/31/13	39,765.00	

[1]Includes direct and indirect costs
*New or competing renewal grants awarded in 2013

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Program Project Support					
Pfizer Inc.	Dr. Stillman	01/01/12	12/31/13	$2,050,000.00	
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lippman/Martienssen/ Timmermans/Ware	07/01/12	06/30/17	1,600,000.00	
The Simons Foundation/Autism	Dr. Wigler	01/01/12	12/31/15	4,000,571.00	
The Simons Foundation/Center for Quantitative Biology	Dr. Wigler	09/01/08	08/31/15	1,500,000.00	
The Stanley Medical Institute	Drs. Watson/McCombie	06/01/11	05/31/17	5,000,000.00	
Research Support					
Alex's Lemonade Stand	Dr. Vakoc	01/01/13	12/31/15	125,000.00	*
Rita Allen Foundation	Dr. C. Hammell	09/01/12	08/31/17	110,000.00	
American Association for Cancer Research	Dr. Egeblad	07/01/12	06/30/14	100,000.00	
Anonymous	Drs. Mitra/Huang	02/01/12	01/31/14	300,000.00	
Anonymous	Dr. Tonks	12/09/13	12/08/14	162,340.00	*
Babylon Breast Cancer Coalition Inc.	Dr. D. Spector	12/30/13	12/29/14	10,000.00	*
Boehringer Ingelheim RCV GmbH & Co. KG	Dr. Vakoc	11/01/13	10/31/15	271,804.00	*
The Breast Cancer Research Foundation	Drs. Wigler/Hicks	10/01/13	09/30/14	240,000.00	*
	Dr. Hicks	10/01/13	09/30/14	154,142.00	*
Burroughs Welcome Fund	Dr. Vakoc	09/01/11	08/31/16	151,250.00	
Caring for Carcinoid Foundation	Dr. Tuveson	07/01/12	06/30/14	255,526.22	
Dysautonomia Foundation Inc.	Dr. Krainer	07/01/12	06/30/14	90,000.00	
Families of S.M.A.	Dr. Krainer	07/01/13	06/30/15	75,000.00	*
Find a Cure Today Long Island Foundation	Dr. Tonks	01/08/13	01/07/14	5,000.00	*
The Joni Gladowsky Breast Cancer Fund	Dr. Tonks	07/01/13	06/30/14	70,000.00	*
Glen Cove C.A.R.E.S., Inc.	Dr. Tonks	02/01/13	01/31/14	5,000.00	*
Global Prostate Cancer Research Foundation	Dr. Wigler	09/01/13	08/31/14	25,000.00	*
The Griffin Fund	Dr. Tuveson	06/15/13	06/14/14	100,000.00	*
The Irving A. Hansen Memorial Fund	Dr. Tonks	08/01/13	07/31/14	25,000.00	*
Hearts for Cancer	Dr. Tonks	04/01/13	03/31/14	35,418.68	*
Robert and Cindy Higginson	Dr. Mills	02/01/13	01/31/14	2,000.00	*
F. Hoffmann-La Roche Ltd.	Dr. Krainer	05/06/13	05/05/15	42,419.00	*
The Hope Foundation	Dr. Egeblad	07/01/12	06/30/14	125,000.00	
Howard Hughes Medical Institute–Gordon and Betty Moore Foundation	Dr. Martienssen	12/01/11	11/30/16	333,333.00	
Ms. Beatrice Karp	Dr. Egeblad	03/01/13	02/28/14	150.00	*
F.M. Kirby Foundation, Inc.	Dr. Vakoc	12/16/13	12/15/14	150,000.00	*
The Susan G. Komen Breast Cancer Foundation, Inc.	Dr. Hicks	10/22/13	10/21/17	246,829.00	*
The Long Island 2-Day Walk to Fight Breast Cancer	Dr. Egeblad	08/15/13	08/14/14	22,500.00	*
Long Island Cruizin' For a Cure Inc.	Drs. Hicks/Trotman	09/01/13	08/31/14	20,000.00	*
The Lustgarten Foundation	Dr. Egeblad	01/01/13	12/31/15	162,883.00	*
	Dr. Tuveson	06/15/12	06/30/17	52,083.00	
	Drs. Tuveson/Pappin	09/01/12	08/30/17	1,000,000.00	
Carol Marcincuk Fund	Dr. Tonks	01/01/13	12/31/13	15,795.00	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2013

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2013 Funding*[1]	
In Honor of Carissa Maringo	Dr. Egeblad	10/01/13	09/30/14	$ 5,282.00	*
Breast Cancer Awareness Day in Memory of Elizabeth McFarland	Dr. Wigler	01/01/13	12/31/13	77,294.20	*
The McKnight Endowment Fund for Neuroscience	Dr. Churchland	07/01/12	06/30/15	75,000.00	
The Melanoma Research Alliance	Drs. Hannon/Hammell/Vakoc	08/01/11	07/31/14	200,000.00	
The John Merck Fund	Dr. Churchland	06/01/11	05/31/15	75,000.00	
	Dr. Kepecs	06/01/10	05/31/14	75,000.00	
The Don Monti Memorial Research Foundation	Drs. Stillman/Tonks	03/01/08	02/28/16	500,000.00	
Louis Morin Charitable Trust	Dr. Tuveson	12/01/13	11/30/14	115,000.00	*
National Philanthropic Trust, Inc.	Dr. Hannon	10/10/13	10/09/14	325,000.00	*
Pancreatic Cancer UK	Dr. Tuveson	10/23/12	10/22/15	65,000.00	
The Pew Charitable Trusts	Dr. Hannon	01/01/11	12/31/15	354,451.00	
	Dr. Albeanu	08/01/12	07/31/17	60,000.00	
Phi Beta Psi Sorority	Dr. Egeblad	08/15/13	08/14/14	58,630.00	*
Pioneer Hi-Bred International, Inc.	Dr. Ware	03/15/12	06/30/15	52,308.00	
The Hazen Polsky Foundation	Dr. Vakoc	01/01/12	12/31/14	50,000.00	
The Prostate Cancer Foundation	Dr. Hicks	09/01/13	08/31/15	184,325.00	*
	Dr. Vakoc	01/20/12	01/19/14	150,000.00	
Christina Renna Foundation Inc.	Dr. Van Aelst	02/01/13	01/31/14	20,000.00	*
Marie Robertson Memorial Fund	CSHL Neuroscience Program Support	01/01/13	12/31/13	137,500.00	*
Diane Emdin Sachs Memorial Fund	Dr. Sordella	09/01/12	08/31/14	9,828.00	
Eleanor Schwartz Charitable Foundation	Dr. Churchland	07/15/11	07/14/14	200,000.00	
The Seraph Foundation	Dr. Vakoc	12/21/13	12/20/14	125,000.00	*
The Simons Foundation	Dr. Mills	08/01/13	07/31/15	208,380.00	*
	Drs. Mitra/Huang	12/01/11	11/30/14	300,000.00	
	Dr. Shea	01/01/13	09/30/13	43,501.00	*
The Sontag Foundation	Dr. Zheng	10/01/13	09/30/17	150,000.00	*
Starr Cancer Consortium	Dr. Vakoc	08/01/11	07/31/13	22,500.00	
Swim Across America	Dr. Sordella	12/15/11	12/14/14	70,000.00	
Tourette Syndrome Association, Inc.	Dr. Lyon	08/01/13	07/31/14	18,454.50	*
Mr. and Mrs. Stanley S. Trotman	Dr. Wigler	11/01/13	10/31/14	2,000.00	*
U.S.-Israel Binational Agricultural Research and Development Fund	Dr. Krainer	10/01/10	09/30/14	10,000.00	
	Dr. Shea	12/01/12	11/30/14	60,000.00	
	Dr. Zador	10/01/10	09/30/14	13,000.00	
The V Foundation	Dr. Vakoc	11/01/12	10/31/14	100,000.00	
West Islip Breast Cancer Coalition for Long Island Inc.	Dr. Tonks	11/01/13	10/31/14	10,000.00	*
Whitehall Foundation, Inc.	Dr. Albeanu	01/01/12	12/31/14	75,000.00	
	Dr. Shea	09/01/11	08/31/15	75,000.00	
Wodecroft Foundation	Dr. Huang	09/27/13	09/26/14	70,000.00	*
	Dr. Wigler	09/27/13	09/26/14	20,000.00	*
Women in Science	Dr. Joshua-Tor	01/01/12	12/31/13	121,576.35	
Elisabeth R. Woods Foundation Inc.	Dr. Sordella	09/27/13	09/26/14	15,000.00	*
Yale University/Gilead Sciences Inc.	Dr. Sordella	07/16/12	07/15/14	407,656.00	
The Bradley Zankel Foundation, Inc.	Dr. Zheng	02/22/13	02/21/14	15,000.00	*
Fellowship Support					
Agricultural Biotechnology Center	Dr. Benkovics	05/01/13	04/30/15	18,862.00	*
American Cancer Society	Dr. Ardito	07/01/13	06/30/16	48,000.00	*
	Dr. Preall	10/01/11	09/30/14	52,000.00	

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Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2013 Funding*[1]	
Brain and Behavior Research Foundation	Dr. Lu	01/15/13	01/14/15	$ 30,000.00	*
	Dr. Paul	01/15/13	01/14/15	30,000.00	*
Terri Brodeur Breast Cancer Fund	Dr. Anczukow	01/01/13	12/31/14	50,000.00	*
Carnegie Institution for Science	Dr. Timmermans	09/01/13	08/31/14	30,482.00	*
CSHL Association Fellowship	Dr. Mills	01/01/13	12/31/13	35,000.00	*
	Dr. Joshua-Tor	01/01/13	12/31/13	245,000.00	*
Marie Curie International Outgoing Fellowships for Career Development	Dr. Hangya	01/01/13	12/31/14	120,148.00	*
European Molecular Biology Organization	Dr. Anselmi	01/01/13	12/31/13	42,355.52	
	Dr. Livneh	01/01/13	12/31/13	55,000.00	
Genentech Foundation	Watson School of Biological Sciences	10/01/12	09/30/15	58,751.00	
German Academic Exchange Service	Dr. Bergmann	09/01/11	08/31/13	11,000.00	
Lola A. Goldring	Dr. Stillman	10/01/13	09/30/14	85,000.00	*
Hope Funds for Cancer Research	Dr. Knott	07/01/12	06/30/15	46,500.00	
Human Frontier Science Program	Dr. Chio	04/01/13	09/30/13	25,860.00	*
	Dr. Kawaoka	11/01/13	10/31/16	61,080.00	*
	Dr. Tam	05/01/13	04/30/14	64,677.50	*
International Rett Syndrome Foundation	Dr. He	02/01/13	01/31/15	50,000.00	*
Annette Kade Charitable Trust	Watson School of Biological Sciences	10/15/13	10/14/14	25,000.00	*
Life Sciences Research Foundation	Dr. MacAlister	08/01/11	07/31/14	56,000.00	
Muscular Dystrophy Association	Dr. Sahashi	02/01/13	02/28/13	4,869.26	*
Mr. and Mrs. John C. Phelan	Watson School of Biological Sciences	09/01/10	08/31/15	100,000.00	
Damon Runyon Cancer Research Foundation	Dr. Chio	10/01/13	09/30/16	52,000.00	*
Damon Runyon Cancer Research Foundation	Dr. Sabin	01/01/12	12/31/14	52,000.00	
Sass Foundation for Medical Research Inc.	Dr. Roe	06/01/13	05/31/15	50,000.00	*
Lauri Strauss Leukemia Foundation	Dr. Mercan	11/15/13	11/14/14	10,000.00	*
The Swartz Foundation	Dr. Zador	01/01/13	12/31/13	75,000.00	*
	Drs. Koulakov/Ferrante	01/01/13	12/31/13	55,000.00	*
	Drs. Churchland/Kaufman	01/01/13	12/31/13	55,000.00	*
	Dr. Zador	01/01/13	12/31/13	15,000.00	*
Training Support					
Clare College	Undergraduate Research Support	06/01/13	05/31/14	6,016.40	*
The Genetics Society	Undergraduate Research Support	05/01/13	04/30/14	30,000.00	*
The Lita Annenberg Hazen Foundation	Undergraduate Research Support	05/01/08	04/30/18	10,000.00	
Howard Hughes Medical Institute	Undergraduate Research Support	06/01/13	05/31/14	17,400.00	*
William Townsend Porter Foundation	Undergraduate Research Support	04/01/13	03/31/14	12,500.00	*
Research Foundation of the City University of NY	Undergraduate Research Support	06/01/13	05/31/14	13,700.00	*
University of Notre Dame	Undergraduate Research Support	06/01/13	05/31/14	30,000.00	*
Course Support					
American Express Foundation	Leadership in Bioscience	11/01/11	10/31/14	50,000.00	
Dr. David Botstein	Yeast Genetics and Genomics	12/01/13	11/30/14	100,413.67	*
Burroughs Wellcome Fund	Imaging Structure and Function in the Nervous System	12/01/13	11/30/14	20,000.00	*
The Gatsby Charitable Foundation	Vision: A Platform Linking Circuits, Perception, and Behavior	06/11/13	06/10/14	10,800.00	*

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2013

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2013 Funding*[1]	
Howard Hughes Medical Institute	Course Support	08/01/11	07/31/15	$ 600,000.00	
Nancy Lurie Marks Foundation	Workshop on Autism Spectrum Disorders	07/01/12	06/30/16	25,000.00	
The Lustgarten Foundation	Workshop on Pancreatic Cancer	01/18/13	01/17/14	15,000.00	*
Pancreatic Cancer Action Network, Inc.	Workshop on Pancreatic Cancer	01/18/13	01/17/14	15,000.00	*
Meeting Support					
Alfred P. Sloan Foundation	New Neuroscience Faculty/ Dr. Goldschmidts	02/01/13	09/01/14	20,000.00	*
Baker Botts L.L.P.	Patenting in the Life Sciences	12/03/12	12/02/13	5,000.00	
Bereskin & Parr	Patenting in the Life Sciences	12/03/12	12/02/13	2,500.00	
Biotechnology Industry Organization	Patenting in the Life Sciences	12/03/12	12/02/13	10,000.00	
Biotechnology Strategy	Mobile Genetic Elements	04/15/13	04/14/14	2,000.00	*
ChemGenes Corporation	RNA and Oligonucleotide Therapeutics	04/01/13	03/31/14	500.00	*
Dart Neuroscience LLC	Mobile Genetic Elements	04/15/13	04/14/14	7,500.00	*
The Ellison Medical Foundation	Asia Conference on Molecular Basis of Aging and Disease	07/01/13	06/30/14	15,775.00	*
Forest Research Institute, Inc.	Wiring the Brain Conference	06/07/13	06/06/14	25,000.00	*
Genentech Inc.	History of Restriction Enzymes	07/24/13	07/23/14	10,000.00	*
	Patenting in the Life Sciences	12/03/12	12/02/13	10,000.00	
Gilead Sciences Inc.	Retroviruses	05/01/13	04/30/14	12,500.00	*
GlaxoSmithKline, LLC	Asia Meeting Support	09/01/13	08/31/14	10,000.00	*
Glenn Foundation for Medical Research	Molecular Genetics of Aging	11/11/13	11/10/14	15,000.00	*
Isis Pharmaceuticals, Inc.	RNA and Oligonucleotide Therapeutics	04/01/13	03/31/14	2,000.00	*
Jones Day	Patenting in the Life Sciences	12/03/12	12/02/13	5,000.00	
Life Technologies Corporation	History of Restriction Enzymes	07/24/13	07/23/14	25,000.00	*
	60th Anniversary of the Double Helix Celebration Gala Dinner	02/08/13	02/07/14	55,000.00	*
	Personal Genomes and Medical Genomics	12/15/12	10/31/14	10,000.00	
Millennium Pharmaceuticals, Inc.	The Ubiquitin Family Meeting	06/01/13	05/31/14	12,500.00	*
Molecular Biology Resources, Inc.	History of Restriction Enzymes	07/24/13	07/23/14	1,000.00	*
New England Biolabs, Inc.	History of Restriction Enzymes	07/24/13	07/23/14	25,000.00	*
Nippon Gene Co., LTD	History of Restriction Enzymes	07/24/13	07/23/14	5,000.00	*
OSI Pharmaceuticals, Inc.	Cancer Biology and Therapeutics	03/01/13	02/28/14	15,000.00	*
Pioneer Hi-Bred International, Inc.	Mobile Genetic Elements	04/15/13	04/14/14	2,500.00	*
Promega Corporation	History of Restriction Enzymes	07/24/13	07/23/14	10,000.00	*
RaNA Therapeutics, Inc.	RNA and Oligonucleotide Therapeutics	04/01/13	03/31/14	4,000.00	*
Regado Biosciences, Inc.	RNA and Oligonucleotide Therapeutics	04/01/13	03/31/14	2,000.00	*
Sarepta Therapeutics, Inc.	RNA and Oligonucleotide Therapeutics	04/01/13	03/31/14	10,000.00	*
Takara Bio, Inc.	History of Restriction Enzymes	07/24/13	07/23/14	10,000.00	*
Thermo Fischer Scientific Inc.	History of Restriction Enzymes	07/24/13	07/23/14	25,000.00	*
Ubiquigent LTD	The Ubiquitin Family Meeting	02/01/11	01/31/14	1,000.00	
Wolf, Greenfield & Sacks, P.C.	Patenting in the Life Sciences	12/03/12	12/02/13	5,000.00	
Library Support					
The Ellen Brenner Memorial Fund		12/15/13	12/14/14	2,500.00	*
Ms. Jolanta Fabicka		01/01/13	12/31/13	5,000.00	*
Institute of Museum and Library Services		10/01/13	09/30/14	50,000.00	*
The New York State Education Department		07/01/13	06/30/14	3,573.10	*
Wellcome Trust		06/01/11	10/31/13	17,500.00	

[1]Includes direct and indirect costs

*New or competing renewal grants awarded in 2013

DNA LEARNING CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2013 Funding*[†]
FEDERAL GRANTS			
National Institutes of Health, University of California, Berkeley	Enhancing the GMOD suite of genome annotation and visualization tools	6/12–5/14	$ 39,330
National Science Foundation, North Carolina State University	GEPR: Epigenome Dynamics during DNA Replication	3/11–2/13	7,617
National Science Foundation, University of Arizona	Educational outreach for *iPlant Collaborative*: A cyberinfrastructure for plant sciences	2/08–7/13	397,011
National Science Foundation, University of Arizona	*iPlant Collaborative*: Cyberinfrastructure for the life sciences	9/13–8/18	363,220
National Science Foundation	Infrastructure and training for next-generation sequence (NGS) analysis in undergraduate education	9/13–8/16	13,752
National Science Foundation	Advanced technology education (ATE) Program: *Genomic approaches in BioSciences*	4/11–3/14	255,955
NONFEDERAL GRANTS			
Alfred P. Sloan Foundation	*DNA Center NYC* start-up	12/13–11/16	9,430
The Pinkerton Foundation	*Urban Barcode Research Program*	1/13–12/14	112,315

The following schools and school districts each contributed $1,000 or more for participation in the Curriculum Study program:

Bellmore-Merrick Central High School District	$1,500	Massapequa Union Free School District	1,500
East Meadow Union Free School District	1,500	North Shore Central School District	3,000
Elwood Union Free School District	1,500	North Shore Hebrew Academy	3,000
Fordham Preparatory School	1,500	Oceanside Union Free School District	1,500
Garden City Union Free School District	1,500	Oyster Bay-East Norwich Central School District	3,000
Great Neck Union Free School District	1,500	Plainedge Union Free School District	1,500
Green Vale School	3,000	Plainview-Old Bethpage Central School District	1,500
Half Hollow Schools Central School District	1,500	Portledge School	1,500
Harborfields Central School District	1,500	Port Washington Union Free School District	1,500
Herricks Union Free School District	1,500	Ramaz Upper School	1,500
Huntington Union Free School District	1,500	Roslyn Union Free School District	3,000
Island Trees Union Free School District	1,500	Syosset Central School District	3,000
Jericho Union Free School District	1,500	West Hempstead Union Free School District	3,000
Levittown Union Free School District	1,500	Yeshiva University High School for Girls	1,500
Locust Valley Central School District	1,500		

The following schools and school districts each contributed $1,000 or more for participation in the *Genetics as a Model for Whole Learning* program:

Alpine Public Schools, New Jersey	1,300	JHS 226, Queens	1,193
Bellmore Union Free School District	2,400	Lawrence UFSD	11,100
Bellmore-Merrick Union Free School District	9,875	Locust Valley Central School District	11,841
Bethpage Union Free School District	2,500	Merrick Union Free School District	3,400
Cold Spring Harbor Central School District	14,400	M.S. 447, Brooklyn	1,450
Commack Union Free School District	6,100	North Bellmore Union Free School District	1,900
East Meadow Union Free School District	4,012	North Shore Central School District	1,750
East Williston Union Free School District	2,900	North Shore Hebrew Academy	1,050
Elwood Union Free School District	3,375	Oceanside Union Free School District	1,625
Floral Park-Bellerose Union Free School District	7,800	Oyster Bay-East Norwich Central School District	2,575
Friends Academy	3,300	Passaic Charter School, New Jersey	1,300
Friends Seminary of New York	1,078	Port Washington Union Free School District	5,500
Garden City Union Free School District	10,355	Rockville Centre Union Free School District	6,240
Great Neck Union Free School District	10,150	Roslyn Union Free School District	3,675
Half Hollow Hills Union Free School District	16,450	Saint Dominic Elementary School	4,550
Hebrew Academy of Nassau County	1,700	Scarsdale Union Free School District	12,600
Herricks Union Free School District	2,975	Smithtown Union Free School District	1,680
Hofstra STEP	1,050	Syosset Union Free School District	38,800
Holy Child Academy	3,200	Three Village Central School District	6,300
Huntington Union Free School District	11,600	Trinity School	2,400
Jericho Union Free School District	7,950	Yeshiva Darchei Torah	2,760

[†]Includes direct and indirect costs.

BANBURY CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2013 Funding*
FEDERAL SUPPORT			
National Institute on Alcohol Abuse and Alcoholism	The Adolescent Brain	2013	$ 4,635
National Institute of Mental Health	The Adolescent Brain	2013	4,635
NONFEDERAL SUPPORT			
Allen Institute for Brain Science	The Adolescent Brain	2013	10,500
ALS Association of Greater New York	Development and Evolution of the Human Motor System in Relation to ALS and FTD	2013	46,367
Boehringer Ingelheim Fonds	Science: Get It Across!	2013	58,594
John K. Castle	Oxidants and Antioxidants in Cancer Genesis and Treatment	2013	20,000
Cold Spring Harbor Laboratory Corporate Sponsor Program	Evolution of Plant Metabolic Diversity	2013	43,732
	Redesigning Photosynthesis: Identifying Opportunities and Novel Ideas	2013	58,988
	Telomeres and Disease	2013	48,358
Cold Spring Harbor Laboratory–DuPont/PioneerCollaborative Research Program	Plant Reproduction	2013	50,000
Dart NeuroScience	Transposable Elements in the Brain and Other Tissues: Prevalence and Function	2013	19,053
Illumina, Inc.	Accelerating Genomic Research with Privacy Protections	2013	34,410
Individual participants	The Adolescent Brain	2013	6,090
Individual participants	Developing a Neuroscience Consortium	2013	23,270
Individual participants	The Emerging Intersection between the Physical Sciences and Oncology	2013	2,060
Individual participants	Grand Challenges in Organismal Biology	2013	1,375
Individual participants	The Neurobiology and Clinical Study of Rapid-Acting Antidepressants	2013	5,500
ISCTM	Developing a Neuroscience Consortium	2013	7,495
Janssen Research & Development	The Neurobiology and Clinical Study of Rapid-Acting Antidepressants	2013	34,362
Kotumba Capital Management, LLC	Science of Pancreatic Cancer	2013	12,572
The Lieber Institute for Brain Development	The Adolescent Brain	2013	12,500
Elizabeth Livingston Estate	Interdisciplinary Approaches to Idiopathic Lung Fibrosis	2013	43,648
Lustgarten Foundation	Lustgarten Foundation Annual Scientific Meeting	2013	38,764
MCJ Amelior Foundation	Science of Pancreatic Cancer	2013	20,060
Oliver Grace Cancer Fund	Oxidants and Antioxidants in Cancer Genesis and Treatment	2013	41,233
Oliver Grace Cancer Fund	Biguanides and Neoplasia	2013	59,632
Oliver Grace Cancer Fund	Enhancer Biology in Health and Disease	2013	51,718
Ovarian Cancer Research Fund	Ovarian Cancer: Developing Research-Based Public Messaging on Early Detection and Screening	2013	31,544
Pfizer, Inc.	INK4a/ARF Network	2013	56,000
Phelan McDermid Syndrome	Autism due to Shank3 Mutations/Deletions Foundation	2013	33,121
Marie Robertson Neuroscience Fund	Transposable Elements in the Brain and Other Tissues: Prevalence and Function	2013	20,000
The Daniel & Joanna S. Rose Foundation	Consciousness and the Brain	2013	10,000

BANBURY CENTER GRANTS (*Continued*)

Grantor	*Program*	*Duration of Grant*	*2013 Funding*
The Satenik and Adom Ourian Educational Foundation	Consciousness and the Brain	2013	$ 1,000
Stanley Research Foundation	Psychiatric Genomics: Current Status, Future Strategies	2013	41,335
Stony Brook University through a grant from NSF	Grand Challenges in Organismal Biology	2013	42,625
University of Southern California NCI Physical Sciences in Oncology Center	The Emerging Intersection between the Physical Sciences and Oncology	2013	27,372

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 2013 as the economic difficulties began to take effect. The year 2014 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*, *CSH Protocols, Genome Research*, *RNA,* and the *Perspective* series.

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 2013 were the following:

CORPORATE BENEFACTORS

Astellas-OSI Pharmaceuticals, Inc.

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DEVELOPMENT

Cold Spring Harbor Laboratory (CSHL) is the birthplace of molecular biology and is home to eight Nobel Prize winners who have made seminal discoveries in genetics. On the cusp of our 125th anniversary, CSHL is launching the Cancer Therapeutics Initiative to extend this broad genetic expertise to clinical trials and drug development. To realize this mission, we have recruited new clinical scientists such as Dr. David Tuveson, who is an expert in pancreatic cancer and has been involved in academic clinical trial development at the University of Cambridge in the United Kingdom. We are grateful to the Lustgarten Foundation and Roy Zuckerberg for supporting Dr. Tuveson's recruitment. It was also essential that we create new infrastructure for this purpose, and through the support of David Koch, New York State, and an anonymous donor, we broke ground in November on the Preclinical Experimental Therapeutics Facility. Together with the planned Center for Cancer & Metabolism, these facilities will greatly accelerate our research efforts aimed at developing and testing new therapeutics and diagnostics for cancer and neurological disorders.

As we continue to strengthen, evolve, and expand our research, CSHL is also extending its educational reach to urban centers and countries all over the world. We are planning our most ambitious public educational undertaking by establishing a flagship center in Manhattan that will serve as the nucleus for DNA learning in New York City. *DNA Learning Center NYC* will exemplify how research institutions and science centers can enhance science education in large cities, by providing an environment in which students and the public can ask questions and perform hands-on experiments. Students and NYC residents will have access to state-of-the-art DNA laboratory experiences in order to gain a greater understanding of their own uniqueness, the implications of personalized medicine, and the genetic heritage that they share with other people in America's melting pot. The vision for the *DNA Learning Center NYC* will be realized with great thanks for a lead contribution by CSHL Trustee Laurie Landeau and with significant gifts by the Thompson Family Foundation and the Alfred P. Sloan Foundation.

These are just some examples of how philanthropy continues to facilitate CSHL in establishing and strengthening its advanced genetics-based initiatives. We look forward to continued partnerships with friends like you on our 125th anniversary and beyond!

Charles V. Prizzi, *Vice President for Development and Community Relations*

CSHL President Bruce Stillman with NYS Economic Development Rep Andrea Lohneiss, NYS Assemblyman Charles Lavine, and LIA President Kevin Law

President's Council

This year's topic was Infectious Diseases and Viruses. CSHL alumni headlined this year's intellectual retreat in September for President's Council members. Nobel laureate and CalTech President Emeritus David Baltimore gave the keynote talk on the latest advances in dealing with the AIDS pandemic. Dr. Baltimore was among the Laboratory's first undergraduate research program (URP) students in the summer of 1959. Dr. Niraj Tolia, a member of the first graduating class of CSHL's Watson School of Biological Sciences (2004), described promising developments toward a protective malaria vaccine. Members also heard from the Gates Foundation President of Global Health, Trevor Mundel, on priority setting; Larry Barrett, Director of the Plum Island Animal Disease Center; Stony Brook Distinguished Professor Eckard Wimmer, who solved the genome sequence for polio and developed the first synthetic vaccine for poliovirus; and Jonathan Epstein of EcoHealth Alliance on his riveting fieldwork to study the ecology of emerging zoonoses.

Loren Eng and Dinakar Singh also hosted Council members and friends in April at their magnificent plaza apartment with sweeping views of Central Park and the Apple store. The lecture, by IBM's Joe Jasinski, focused on the growing role of Internet technology in tracking and preventing virus outbreaks.

I am grateful to our many President's Council members who have, since 1995, helped to support CSHL fellows with annual gifts of $25,000 or more. CSHL fellows are outstanding young scientists who are given the opportunity to pursue a period of independent research soon after receiving their Ph.D. or M.D. degrees.

Bruce Stillman, Ph.D., F.R.S.
President and Chief Executive Officer

CSHL president Bruce Stillman (*left*) with Trevor Mundel, Gates Foundation president of Global Health, and Eckard Wimmer, Stony Brook University

CSHL Trustee Jim Stone with his wife, Cathy

Peter Klein, Liz Murray, and Nick Richardson

Cold Spring Harbor Laboratory Association

With more than 1200 members, the Cold Spring Harbor Laboratory Association continues to increase community awareness of CSHL and this year helped to raise more than $6.5 million in unrestricted support for the Laboratory. Events spearheaded by Directors of the Association included the Women's Partnership in Science luncheon, where Liz Watson was honored for her devotion to CSHL and the community, and the 20th annual golf tournament at Piping Rock Club, where Paul Amoruso was the honoree. The Association was also instrumental in the success of the Double Helix Medals Dinner in New York City with honorees including newscaster Robin Roberts and the founders of the Innocence Project, Barry Sheck and Peter Neufeld.

Special thanks go to Sandy Tytel, President of the CSHL Association, for her support of and enthusiasm for Cold Spring Harbor Laboratory.

Directors

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Sandy Tytel, *President*
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2013 CSHLA Directors

Cold Spring Harbor Laboratory Corporate Advisory Board

The Corporate Advisory Board (CAB) is a vital liaison between Cold Spring Harbor Laboratory and the tristate business community. Established more than 20 years ago, the CAB is the driving force behind the Laboratory's annual golf outing at Piping Rock Club; this outing raises critical unrestricted funding for research and educational programs. At this year's 20th annual event, CAB Member Paul Amoruso was honored and more than $175,000 was raised.

Special thanks go to Eddie Chernoff, CAB President and Golf Chairman, who continues to provide leadership and support. We truly appreciate all of his efforts.

Corporate Advisory Board
Chairperson: Edward A. Chernoff, MARS

Michael Aboff, Aboff's Inc.
Paul Amoruso, Oxford & Simpson Realty
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Nancy Szigethy, NMS Management
Edward Travaglianti, TD Bank
Andrew Whiteley, Enzo Life Sciences
Joseph M. Willen, Advantage Title
Hans Zobel

CSHLA Director Whitney Posillico with her three sons at CSHL's golf tournament

CAB Chairman Eddie Chernoff (*left*) with 2013 Golf Tournament honoree Paul Amoruso (*center*) and CSHL President Bruce Stillman

Honor Roll of Donors

Contributions of $5 million+ lifetime

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The Arnold and Mabel Beckman Foundation
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Contributions of $100,000 or $1 million+ lifetime

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Angel Foundation
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Dr. and Mrs. Lalit Bahl
BioBay of Suzhou Venture Capital Group
Drs. Renee Fitts and David Botstein
Bristol-Myers Squibb Company
Burroughs Wellcome Fund
Louise Parent and John Casaly
The Chapman Foundations
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Jacob Goldfield
Lola Goldring

Deborah Norville, Lee and Elizabeth Ainslie, Bruce Stillman, Terry Lindsay, and Bill and Susan Sheeline

Kinematica CEO Andreas Niens (*second from right*), with CSHL researchers accepting an autoclave donation

The Florence Gould Foundation
The Grace Family
Michael J. Griffin
Kate Medina and Leo Guthart
The Harrison Foundation and The Mary Anderson Harrison Foundation
Thomas Hartman Foundation for Parkinson's Research
Janet Strauss and Jeff Hawkins
Jo-Ellen and Ira Hazan
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Whitehall Foundation, Inc.
The Roy J. Zuckerberg Family Foundation

2013 Double Helix honorees Peter Neufeld, Robin Roberts, and Barry Scheck with CSHL President Bruce Stillman

Contributions of $50,000+

Anonymous (2)
Ellen and Casey Cogut
Joni Gladowsky Breast Cancer Foundation
Mr. and Mrs. David M. Knott
Betsy and Bryan H. Lawrence
Life Technologies
Mr. and Mrs. Edward A. Chernoff–MARS
Breast Cancer Awareness Day in Memory of Elizabeth McFarland
Pershing Square Foundation
Hope Geier Smith and Theodore Smith III
Swim Across America Nassau/Suffolk
Mr. and Mrs. Peter S. Tilles–Peter S. and Doris M. Tilles Foundation
Wodecroft Foundation

Contributions of $25,000+

Elizabeth and Lee Ainslie
The Bahnik Foundation
Hans and Pien Bosch
Broad Hollow Bioscience Park
The Carson Family Charitable Trust
Julia Coleman
Constance and Yves de Balmann
Mr. and Mrs. Geoffrey de Lesseps–Alpha/Omega Charitable Foundation, Inc.
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Mona Spector
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Adjunct Professor

Michael Zhang

Adjunct Assistant Professor

Robert Lucito

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Katherine Borges
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Edu Flores Sandoval
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Marcin Mycko
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Angelina Vaseva
Son Lang Vi
Fang Yang
Jianchao Yao
Zhu Zhu

Computational Science

Assaf Gordon
Manasa Kolli
Michael Malave
Jason O'Rawe

Research Associate

Zsolt Lazar

*Employee of the Howard Hughes Medical Institute