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Basic research is a central, defining activity of Cold Spring Harbor Laboratory. It is the wellspring of both new knowledge and technological advances that make new discoveries possible.

This discovery science is expensive, and getting more expensive every year. Inflation in the biomedical sector outpaces that of the broad U.S. economy, mostly because of the wide use of advanced technologies that require expensive equipment or reagents. We also pay a premium for the highly trained personnel who are needed to offer ever increasingly high-tech methods to our faculty, postdoctoral fellows, and students. At the same time, we continue to see an erosion of the total amount of support any highly meritorious scientist can obtain from federal sources such as the National Institutes of Health (NIH) and the National Science Foundation (NSF). At the NIH and NSF, policies implemented in the last 4 years have deliberately limited the type and amount of funding that can be awarded to the nation’s very best and most productive scientists in order to “spread the grants as widely as possible,” a form of scientific socialism that does not bode well for the future of U.S. science. At the same time, however, opportunities abound in many areas of science, including cancer, neuroscience, plant biology and quantitative biology—areas of focus at Cold Spring Harbor.

Fortunately, with very strong support from our Board of Trustees and supporters of CSHL, we have seen a dramatic increase in our endowment. But this precious resource should support the core of what makes CSHL one of the leading research institutions in the world of basic discovery science. Having recognized this, it has been increasingly obvious that there are many instances in which we can add value to our science and translate these discoveries so they will make an impact in the clinic—and this is particularly the case for cancer.

With this background, we took the initiative in 2015 of entering into a strategic affiliation with Northwell Health, previously known as the North Shore-LIJ Health System. It is an alliance that I expect to be transformative. It will provide an unprecedented opportunity to add value to certain of our discoveries and multiply the impact of our research.

The sweet spot of the affiliation is translational cancer research, which includes the development of new cancer diagnostics and therapeutics and the training of a new generation of research-capable cancer clinicians. Northwell Health is one of the largest integrated health systems in the nation. Its recently expanded Cancer Institute, with more than 200 academic oncologists and clinicians, is part of a system of care encompassing 21 hospitals and 400 outpatient physician practices throughout the New York metropolitan area. Serving more than 8 million people, Northwell treats some 19,000 new cancer cases annually. This makes it one of the most important sites of cancer treatment in the United States.

As CEO Michael Dowling has noted, Northwell’s oncologists will make CSHL’s most promising preclinical research available to cancer patients in the form of innovative trials. Patients will be receiving advanced treatments and diagnostics they would not otherwise be offered and benefit from them years before they would be available to patients elsewhere. At the same time, Northwell’s large patient intake provides our scientists with opportunities to perform cancer research using tumor samples from precisely defined subsets of patients. As we move further into the era of targeted therapy, assembling appropriate patient cohorts becomes ever more critical if we want to speed the time it takes to evaluate new treatments.

Clinician–scientists at Northwell have already begun teaming up with faculty at CSHL. Each team has a specific disease focus or a focus within broad types of cancer such as particular subtypes of breast or prostate cancer. Under the leadership of Dr. David Tuveson, deputy director of CSHL’s National Cancer Institute (NCI)-designated Cancer Center and a talented clinician–scientist, Northwell–CSHL teams have begun to gather periodically at our Banbury Center to...
plan and assess their work. Our agreement additionally supports the education and training of Oncology Fellows. In this aspect of the alliance, the clinical training of oncologists in the Northwell Health system, in conjunction with the Hofstra Northwell School of Medicine, will include an elective period of laboratory research at CSHL. Via summer and full-year fellowships, a cadre of cancer doctors in training will emerge to play an active role in translating the next wave of fundamental discoveries about cancer into new diagnostics and therapies.

It is reasonable to ask how the new alliance will change the way research is done at CSHL. I want to make clear that it in no way alters our core commitment to basic research, which is unshakable. This collaboration adds to our capabilities in a manner illustrated by two contrasting stories about basic research. One of these stories came to a happy conclusion early in 2015 when the Food and Drug Administration approved palbociclib (Ibrance) for the treatment of metastatic breast cancer. It is a first-in-class inhibitor of CDK4 and CDK6, enzymes called protein kinases that help regulate the cell division cycle. In 1991, David Beach, then a highly productive CSHL basic scientist who had already published many papers helping to identify the molecular players involved in the control of cell division, reported the discovery of yet another factor, which he called Cyclin D. This discovery coincided with the same finding by former CSHL Trustee Charles Sherr of St. Jude Children’s Research Hospital, who went on to discover the protein kinase CDK4, which forms a complex with Cyclin D. It soon became clear from the work of Beach and Scherr that the Cyclin D–CDK4 complex is a critical node in the fundamental decision of whether a cell keeps dividing or rests from proliferation. Importantly, it became apparent that most cancer cells have mutations in this control pathway, thereby pushing tumor cells on the road to unchecked growth and aggressive cancer.

What is poignant about the 2015 approval of Ibrance is the fact that it came a quarter-century after the fundamental discoveries by Beach and Scherr. In the early 1990s, we simply did not know enough about cancer to convert their newly generated knowledge into an effective anticancer drug. Now we do. Another basic research discovery at CSHL, this one quite recent, makes the point about our progress vividly, suggesting why the time is ripe for a clinical alliance that enhances our ability to promptly take our basic insights into the clinic. In 2011, Christopher Vakoc, Scott Lowe, and CSHL colleagues performed a screen using RNA interference (RNAi) technology developed at CSHL by Gregory Hannon and his team. The 2011 discovery, which Vakoc has carried forward, revealed a drug target—a protein called BRD4—of unusual potential in the treatment of aggressive forms of leukemia called acute myeloid leukemia (AML). Vakoc discovered that a drug—developed for another purpose by collaborating scientists at the Dana Farber Institute—hit the target, virtually eliminating AML in mouse models. These studies induced a number of pharmaceutical and biotech companies to initiate clinical trials that target AML, some of which are now in Phase II, with positive results already reported from Phase I studies. This is precisely the kind of rapid translation of an important basic scientific result that our new alliance with Northwell Health and its vast clinical system is designed to facilitate. It will enable us to pursue translational science with a vigor we otherwise could not while keeping our basic discovery engine primed.

Two members of our faculty are now reaping the rewards of decades of meticulous basic research. Adrian Krainer’s research on RNA splicing—which began in the 1990s and grows out of earlier Nobel Prize–winning work by Louise Chow and Richard Roberts at CSHL and by Sue Berget and Phillip Sharp at MIT—has made possible the development of a drug, now in Phase III trials, for the serious children’s disease, spinal muscular atrophy (SMA). Nicholas Tonks’ fundamental discovery 25 years ago of the first of what proved a large family of enzymes called protein tyrosine phosphatases (PTPs) was the beginning of a scientific odyssey in which Nick has persisted in the face of doubters in the pharmaceutical industry. Tonks’ team has recently demonstrated their ability to target PTP1B—with a drug Nick developed years ago—in cellular
signaling pathways that play a key role in HER2-positive breast cancer. Phase I trials will begin at Northwell in the spring of 2016. Other PTP1B-targeting compounds in Tonks’ lab are being evaluated by a major pharmaceutical firm for treatment of diabetes and obesity. It is another illustration of how basic science can pay off in ways that are not contemplated at the outset. We see similar promise in other fields: for instance, in Zachary Lippman’s basic research on the process of branching morphogenesis in plants, which now points to a way of significantly increasing fruit yields, and in Steven Shea’s fundamental research on social behavior in rodents, which has led to unexpected insights into Rett syndrome, an autism spectrum disorder.

Basic research has made all of these opportunities possible. To keep our discovery science robust, we were pleased this past year to have been asked to join the ranks of select institutions named as beneficiaries of the Scientific Philanthropy Alliance (SPA). The SPA serves as an impartial advisor to major philanthropists, promoting basic research as the driver of new ideas and of new economic wealth and for the education of a new generation of talented new scientists. Several benefactors of the Laboratory organized the SPA, which we thank for providing another line of support for the basic research that is the lifeblood of Cold Spring Harbor Laboratory.

Bruce Stillman, Ph.D., F.R.S.
President and Chief Executive Officer
Highlights of the Year

Research

In 2015 Cold Spring Harbor Laboratory celebrated its 125th year. Today’s CSHL is renowned for its research in Cancer, Neuroscience, Plant Biology, Quantitative Biology, and Genomics. Scientists at the Laboratory work together, frequently across disciplines, to solve biology’s most challenging problems. This collaborative spirit and the scope of the faculty’s research interests are suggested in this sample of a few of the past year’s important findings.

Organoids to Aid Pancreatic Cancer Research

All cancer research relies on a steady supply of cells, both normal and cancerous, that can be grown in the laboratory. By comparing normal cells to cancer cells, scientists can identify changes that lead to disease. Yet both types of pancreatic cells have been difficult to culture in the laboratory. Another problem in studying pancreas cancer is the fact that many patients when diagnosed are already beyond the point at which surgery is useful, making it difficult or impossible in many cases to obtain tumor samples. To address this, CSHL’s David Tuveson, in concert with the Dutch scientist Hans Clevers, has developed a method to grow pancreatic tissue directly from cells sampled from cancer patients. The cells form tiny spheres called organoids that are entirely made up of ductal cells, eliminating the surrounding cell types that often contaminate samples from the pancreas. They grow within a complex gel-like substance filled with growth-inducing factors and connecting fibers. Once they have grown to a sufficient size, the organoids can be transplanted into mice, where they recapitulate human pancreatic cancer. The Tuveson lab has used organoids to interrogate new therapeutic targets in pancreas cancer; David Spector’s lab has used a similar method to study targets in breast cancer. The hope is that by testing drugs in these three-dimensional hollow spheres made of tumor cells and comparing them to a patient’s normal cells—which can also be grown into organoids—we will be able to much better predict patient treatment outcomes in the clinic.

Biomarker for Treatment-Resistant Prostate Cancer

In 2015 a new animal model for prostate cancer called RapidCaP emerged from Lloyd Trotman’s lab. It is the only model in mouse in which the cancer metastasizes to the bone. This is precisely what happens in advanced metastatic prostate cancer. It is crucial to have such a model, because patient responses to hormone therapy vary widely, and it is still unclear why some types of prostate cancer seem to be resistant to the therapy. Those cases that resist therapy—a minority—are liable to become metastatic and are often lethal. Crucially, Trotman’s model may help us determine which ones. His team has been using this system to trace the mechanisms underlying metastatic lesions. So far they have discovered that such lesions are very different from primary tumors in the prostate. Their work has shown that these metastases activate a pathway that involves the interleukin-6 (IL-6) protein, which activates the MYC oncogene that is expressed specifically in therapy-resistant metastatic prostate cancer cells. Using the IL-6 marker or associated proteins to predict which patients would benefit from hormone therapy would be a major advance. The hope is that translating the IL-6 discovery into clinics could help stratify patients into good responders and bad responders.

Brain Circuit Implicated in Schizophrenia

The prefrontal cortex (PFC) plays an important role in cognitive functions such as attention, memory, and decision-making. Faulty wiring between the PFC and other brain areas is thought to
give rise to a variety of cognitive disorders. Disruptions to one particular brain circuit—between the PFC and another part of the brain called the thalamus—have been associated with schizophrenia, but the mechanistic details are unknown. Bo Li and colleagues have now discovered an inhibitory connection between these brain areas in mice that can control the timing of information flow into the PFC. This insight may help explain what goes wrong in schizophrenia and indicate a path to new treatments. The team used optogenetic stimulation, a technique in which neurons expressing a light-sensitive protein are controlled with pulses of light, to observe a process called feed-forward inhibition, a mechanism in which one neuron excites a neighboring or "downstream" neuron but also recruits a third neuron to inhibit the downstream target after some delay. They will now use a genetic mouse model of schizophrenia to determine if there are any noticeable changes in the observed feed-forward inhibition in the thalamus–PFC pathway; these in turn might suggest novel targets for next-generation schizophrenia therapeutics.

Cholinergic Warning System

In experiments with mice, Adam Kepecs and colleagues discovered a set of dedicated neurons in the basal forebrain that broadcast messages throughout the cerebral cortex, rapidly informing multiple distributed subregions of any surprising rewards or punishments. The neurons are cholinergic—they send signals in the form of the neurotransmitter acetylcholine. Such neurons are thought to play an important role in arousal, attention, and learning, yet their precise role in behavior has remained mysterious, in part because of the technical difficulty in recording from them in vivo. Degeneration and loss of cholinergic neurons in the basal forebrain have been implicated in Alzheimer's disease, age-related cognitive decline, and other cognitive disorders and dementias. Kepecs' team showed how central cholinergic neurons function, using optogenetic methods as mice performed behavioral tasks that involved rewards or unexpected mild punishments. To explain their responses the team constructed a computational model that revealed that the modulation of signal strength was proportional to how unexpected or surprising the mice found the reward or punishment. According to the model, if the mice were confident their response was correct, the reward generated a weak signal. But if they were unsure, the reward came as more of a surprise and generated a stronger cholinergic signal. Kepecs suggests that cholinergic broadcasts to the cortex would be useful in boosting plasticity, allowing flexibility in neuronal connections that makes learning possible. Whether a surprise is positive or negative, the fact that it is unexpected, and the degree to which it is, would be an obvious advantage to the individual.

How a Brain Circuit Controls Fear

It is hard to imagine that an intangible emotion like fear is encoded within neuronal circuits, but Bo Li and colleagues have found that fear is stored within a distinct region of the brain. In recent years, they have discovered that fear learning and memory are orchestrated by neurons in the central amygdala. Now, Li, along with CSHL collaborators Josh Huang and Linda Van Aelst, took on the question of what controls the central amygdala. They focused on a cluster of neurons that form the PVT, or paraventricular nucleus of the thalamus. This region is extremely sensitive to stress, acting as a sensor for both physical and psychological tension. The team found that the PVT is specifically activated as animals learn to fear or as they recall fear memories. They were able to see that neurons from the PVT extend deep into the central amygdala. Disrupting the connection significantly impaired fear learning. Perhaps most important, in looking for molecular mechanisms that connect the two structures, the team...
used data from people with post-traumatic stress disorder to discover that the well-known neural growth factor BDNF is the chemical messenger that allows the PVT to exert control over the central amygdala. This provides a promising target for the future treatment of anxiety disorders.

**Mastering CRISPR to Reveal Cancer Targets**

Since assembly of the full human genome sequence in the early 2000s, scientists have been amassing databases that document or predict how specific stretches of DNA letters in the genome encode specific segments of proteins, called domains. Among the domains of greatest interest to drug developers are those that form pocket-like features on the surface of proteins that other molecules can fit into, as keys fit into locks. Drugs are keys that fit into binding-pocket locks, sometimes for the purpose of blocking access to the lock, and other times to initiate a cascade of signaling inside the cell. Watson School doctoral student Junwei Shi spent a year in Chris Vakoc’s lab mastering the new genome editing method called CRISPR-Cas9, which has taken the world of biology by storm. He and Vakoc used the technique to mimic the effect of drugs binding to protein targets on leukemia cells—thousands of different targets in a single experiment. This multiplexing approach yielded exciting results, successfully identifying six previously known targets and revealing 19 previously undiscovered ones in just one leukemia screen. Each one is a binding pocket that if blocked by a drug would result in cell death—in other words, a target that the cancer cell depends on to thrive. It was a proof of principle. The method can be used by academic labs and by drug developers to generate catalogs of the most effective druggable targets for cancer cells, across different cancer types and subtypes.

**Our Probabilistic Approach to Numbers**

Humans, including preverbal babies and adults in indigenous cultures with no formal mathematical education, are capable of estimating numbers of objects. Yet although areas of the brain have been identified that respond to specific numbers, it has been unclear how numbers are represented. Scientists have generally assumed that the brain represents numbers of objects as single, whole values, or “scalars.” However, estimates of many other features of the environment—such as object depth, height, and location—have been shown to be “probabilistic,” represented as a range of values. In 2015 Anne Churchland and colleagues reported results of an experiment combining auditory and visual cues to test whether people have a scalar or probabilistic sense of numbers. They determined that even a distinct number of objects in the world may be represented in the brain not as a single value but as a range of possible values. Subjects could perform an audiovisual task involving a numerical determination with any of three strategies. Some employed only the most reliable piece of information; others combined auditory and visual information to arrive at an estimate; still others randomly picked one piece of information on which to base their number estimate. These results have important implications for how we learn and understand our world. Representing numbers as a range of possible values allows people to utilize multiple streams of information, leading to improved decisions.

**Reversibility of Rett Syndrome Symptoms**

Another example of collaborative science at CSHL is newly published work that is the fruit of discussions among members of the laboratory groups of Nicholas Tonks, Stephen Shea, and Josh Huang. The idea behind Tonks and colleagues’ 2015 report of an experimental drug treatment to reverse symptoms of Rett syndrome, an autism spectrum disorder, can be traced to a discussion among investigators working in the three
labs. They realized there might be some benefit in applying to a mouse model of Rett syndrome some of the work that’s been done in the Tonks lab in developing small-molecule drugs that inhibit an enzyme called PTP1B, which Tonks discovered 25 years ago. Realizing that metabolic regulation appears to be abnormal in Rett syndrome—a largely unappreciated fact—Navasona Krishnan, a Research Associate who works with Tonks, proposed using inhibitors of PTP1B to see if they might address any of the range of symptoms seen in the disease. He first demonstrated that PTP1B levels were abnormally elevated in the model mice. This was an encouraging sign that inhibitors of PTP1B might have a beneficial effect. More exhaustive experiments with several candidate small-molecule inhibitors demonstrated that they can significantly extend life span in male mice that model Rett syndrome and can ameliorate several behavioral symptoms of the disorder in model female mice. This was tantalizing evidence that Rett symptoms can actually be reversed and supports the concept that the disorder may be amenable to treatment with small molecule drugs—an objective the team continues to pursue vigorously.

Fine-Tuning Plant Growth to Optimize Fruit Size

A wonderful example of basic science having an important societal impact is work from Zach Lippman’s laboratory. His discoveries in recent years have identified a number of things that can be done by growers to increase fruit yield. This year Lippman and colleagues identified a set of genes that control stem cell production in tomato. Mutations in these genes explain the origin of mammoth beefsteak tomatoes. More important, the research suggests how breeders can fine-tune fruit size in potentially any fruit-bearing crop, a significant advance for agriculture. The secret of the beefsteak tomato, the team showed, has to do with the number of stem cells in the plant’s growing tip, called the meristem. They traced an abnormal proliferation of stem cells to a naturally occurring mutation that arose hundreds of years ago in a gene called CLAVATA3. Selection for this rare mutant by plant cultivators is the reason we have beefsteak tomatoes today. Lippman’s team examined never-before-studied mutant tomato plants, three of which contained faulty genes encoding enzymes that add sugar molecules to proteins. Their experiments revealed that the enzymes, called arabinosyltransferases (ATs), add sugar molecules called arabinoses to CLAVATA3. By adjusting the number of sugars on CLAVATA proteins, and through other mutations affecting components of the pathway, Lippman and colleagues show it is possible to fine-tune growth in ways that could allow breeders to customize fruit size.

Overcoming Bad Karma

Epigenetics pioneer Rob Martienssen, whose discoveries confirm and extend the observations and predictions of CSHL Nobel laureate Barbara McClintock, this year solved a 30-year-old mystery that had cost growers of the oil palm tree hundreds of millions of dollars in ruined crops. In the 1980s, a new method of generating plantations brimming with clones of the highest-yielding specimens of the oil palm plant met with unanticipated disaster. Corporate investors were astonished to observe that the finest hybrids, cloned in culture dishes, often grew into barren adults bearing desiccated, worthless fruits. These plants displayed a mutant form that scientists called “mantled.” Martienssen’s work, aimed at more completely understanding how epigenetic mechanisms influence and even control plant development and evolution, traced the problem to a transposable element lodged within the oil palm gene called MANTLED. This “jumping gene” is an example of the myriad genomic invaders that lay (mostly) dormant within and between genes in all forms of life. This particular invader, or one very similar to it, was first spotted in rice plants and had been named karma. Martienssen and colleagues discovered that in mantled plants, a methyl mark present in healthy plants was missing at a location in karma called a splice site.
When the splice site is unmethylated, the RNA message copied from the gene encodes a mutant protein that gives rise to plants with worthless fruit. A simple epigenetic test will readily identify bad *karma* and thus enable growers to cull damaged clones at the plantlet stage. It will promote the propagation of healthy high-value hybrid clones and thus reduce the economic pressure on growers large and small to devote additional tropical rainforest territory to oil palm cultivation.

**1890–2015: The Laboratory’s 125th Anniversary**

July 7, 1890 marked the first day of the first course ever given at The Brooklyn Institute’s Laboratory of Biological Research at Cold Spring Harbor. The first scientists to come to learn and research at Cold Spring Harbor paid $24 in tuition and were encouraged to bring their own microscopes. We celebrated the 125th anniversary of this day—the start of what is today CSHL—continuously throughout the year with a diverse set of events designed for our own campus community, the broader scientific community, and the public.

More than 600 people visited the Bungtown Road campus on June 6 for a public celebration of the 125th Anniversary. The Open House featured stations representing all research and education divisions in “expo-style” format that encouraged visitors to interact with our faculty, students, and staff.

Especially popular were campus mini-tours running throughout the day, which took more than 300 guests through campus and were led by Ph.D. students and postdoctoral researchers. Kids and adults alike crammed booths where DNA Learning Center teachers led hands-on biology demonstrations, featuring DNA extraction and mutant fruit flies. All of CSHL’s research areas were represented by science researchers with hands-on and interactive stations in the just-completed Nicholls Biondi Hall. A special treat for the event was a painting of the CSHL campus landscape commissioned privately by Dill Ayres. It was on display with its creator, famed local painter William Jonas, who spoke to visitors about the painting. We were also pleased to host the Cold Spring Harbor High School Research Symposium as part of the day’s events, featuring CSHL Professor and Lustgarten Foundation Director of Research Dr. David Tuveson, who delivered the keynote, *Avoid Boring Problems.*
Many thanks to all of the faculty, students, and staff who made the Open House such a special day for so many in our community. We also appreciate the year-long efforts of many of our graduate students and postdoctoral fellows who serve as ambassadors to the community, guiding our public tours on weekends and for visiting civic, student, and other organizations. Over the year, more than 1500 visitors were treated to these professionally guided tours on the history, contemporary science, and education programs of CSHL.

A special anniversary public lecture series also brought scientific luminaries to the Lab throughout the year. (See the calendar of these events listed at the end of this section.) To commemorate and share our anniversary more widely through print and digital channels, the Public Affairs Department developed an interactive digital timeline of CSHL’s historic contributions to society (discoveries.cshl.edu) and the CSHL Press published *The Road to Discovery: A Short History of Cold Spring Harbor Laboratory* by our own Banbury Center Executive Director Jan Witkowski.

I also hope that our faculty, students, and staff enjoyed the many celebratory events on campus, designed and executed by a volunteer committee. Congratulations, too, to all of the winners of competitions that included, a raft race, karaoke, CSHL trivia, and a scavenger hunt!

**CSHL-North Shore LIJ Health System (Northwell Health) Affiliation**

On April 5, CSHL and Northwell Health (previously the North Shore-LIJ Health System) announced a strategic affiliation to align the Lab’s world-class cancer research with Northwell’s growing network of clinical services encompassing more than 16,000 new cancer cases annually across the New York metropolitan area.

The CSHL-Northwell affiliation will benefit from the investment of more than $120 million to accelerate cancer research, diagnosis, and treatment. Funds will be used to advance cancer therapeutics research, develop a new clinical cancer research unit at the North Shore-LIJ Cancer Institute’s headquarters in Lake Success, New York to support early-phase clinical studies of new cancer therapies and recruit and train more clinician–scientists in oncology.
It is a transformative affiliation for both institutions, bringing the cutting-edge basic discovery science and translational cancer research at CSHL to one of the largest cancer treatment centers in the United States. The unique integration of research scientists, clinical translational researchers, and cancer clinicians promises to speed the advance of novel cancer diagnostics and therapeutics to patients in the region.

The affiliation will recruit cancer researchers and clinicians to the region. As part of the collaboration, clinician–scientists will be trained to perform preclinical cancer research and conduct early-stage human clinical trials. Positive findings from this partnership in research and therapeutics will form the basis for advanced-phase clinical trials to be conducted at both Northwell facilities and collaborating outside medical centers. Patients cared for at Northwell facilities will benefit from increased access to these innovative clinical studies.

Under the terms of the strategic affiliation, Northwell and CSHL will continue as independent organizations governed by their respective Boards of Trustees. A committee with responsibility for oversight, staffing, and implementation of the affiliation includes three representatives of CSHL: President and CEO Dr. Bruce Stillman, Director of Research Dr. David L. Spector, and Cancer Center Deputy Director Dr. David Tuveson; and three representatives of North Shore-LIJ: Physician-in-Chief and Dean of the Hofstra-Northwell School of Medical School Dr. Lawrence G. Smith, Feinstein Institute for Medical Research President and CEO Dr. Kevin J. Tracey, and Dr. Thomas McGinn, Chair of Medicine.

**Economic Impact of the Lab**

The Laboratory commissioned a study by the private consulting firm Appleseed of its direct and indirect impact on the Long Island and New York State economies. Based on 2013 data, *Shaping Long Island’s Bioeconomy: The Economic Impact of CSHL* highlighted the institution as an enterprise directly employing 1100, indirectly accounting for more than 500 additional jobs, and annually bringing more than $140 million in revenue to Long Island from Federal grants, private philanthropy, an array of scientific educational programs, and commercialization of technologies. The private, not-for-profit Laboratory’s research revenue in 2013 totaled $104.3 million, an increase of 65% since 2003.

The study found that CSHL “to an extent few other institutions can match” is equipped to address the demands of what the National Research Council (NRC) calls “the new biology.” This involves integrating insights across normally “siloed” disciplines, ranging from information and computer technologies to physics, and putting them to work to solve pressing problems, from new approaches to treating cancer and disabling brain disorders to developing insights that result in increased food production and the generation of clean, low-cost biofuels.

Furthermore, the study concluded that the impact of the Lab is multiplied through its collaborations with other New York institutions and companies, including Stony Brook University and Brookhaven National Laboratory in neuroscience and biofuels, and with health-care institutions like Northwell Health. Partnerships with biotechnology and pharmaceutical companies such as GSK, Pfizer, Boehringer Ingelheim, Dart Neurosciences, Isis Pharmaceuticals, Merck, and Syngenta enable the Lab to speed the process of turning research developments into tangible benefits to society.
Business Development & Technology Transfer

The Business Development & Technology Transfer program supports the Lab’s scientists with industry and investor engagements in seeing CSHL discoveries reach the public. In 2015 we added several new technology projects and 18 new patent filings bringing the portfolio under management to 142 technologies covered by more than 500 active patent cases. Eleven new industry-sponsored research or collaboration agreements were signed and 21 new license and option agreements were signed, including:

• the GSK DPAc research collaboration initiated to identify new therapies for metabolic diseases
• Hairpin LLC, launched as our licensing agent for the shRNA technology and a platform for distribution of enabling research technologies developed at CSHL
• research collaborations with Pfizer, BI, and Syros in Chris Vakoc’s CRISPR innovations
• the exercising of ASOthera Pharmaceuticals’ option to a full license with equity and transitioned to a real company, well financed with excellent management
• Marvel Genomics, our newest spin-out, off to a good start with an energetic start-up CEO, an experienced executive in the CSHL board seat, and two corporate partnerships in discussion

Research Faculty

Awards

Evelyn Witkin, Ph.D., who worked as a geneticist here in the formative years of molecular biology, was honored at age 94 with a Lasker Award for achievement in Basic Science. Evelyn made her mark in biology by identifying mechanisms of DNA repair and recombination through experiments on bacteria. (See a detailed account of her career at the CSHL Library & Archives website.)

She earned her Ph.D. in 1947 with Theodosius Dobzhansky at Columbia University, but she was directed by Dobzhansky to study with then—CSHL Director Milislav Demerec where her interests turned to bacterial genetics, and she spent the summer of 1944 at Cold Spring Harbor, where she isolated a radiation-resistant mutant of Escherichia coli. Evelyn continued her studies on the shores of Cold Spring Harbor as part of the Carnegie Institution Department of Genetics until 1955. Subsequently she took a position at the State University of New York’s Downstate Medical Center in Brooklyn, and in 1971 became a professor at Rutgers University. She was named Barbara McClintock Professor of Genetics in 1979 and was appointed in the Waksman Institute at Rutgers in 1983. Evelyn retired in 1991.

Professor Anthony Zador was recognized by the editors of the prestigious journal Foreign Policy in the category of “Innovators,” for “testing the boundaries of the brain, our most powerful organ,” according to the journal. Others receiving “Top 100” honors in recent years have spanned the gamut of achievement, from Pope Francis to Angela Merkel to Elon Musk. Tony was recognized for his work on developing a wiring diagram of the mouse brain at the resolution of individual neurons.

Professor Marja Timmermans, a distinguished plant geneticist who has spent most of her career at the Lab, began a new phase of her work in April, as an Alexander von Humboldt Professor at Germany’s Tübingen University. Germany’s most prestigious international research prize was presented by that nation’s Research Minister, Professor Johanna Wanka, and President of the Humboldt Foundation, Professor Helmut Schwarz. Marja has made several internationally acclaimed discoveries. She has explained key mechanisms behind leaf development in plants and, most recently,
demonstrated the mobility of small RNA molecules and how this establishes leaf polarity—the process through which leaves flatten and develop distinct top and bottom surfaces optimized for photosynthesis. She began her career at CSHL in 1998 and was named assistant professor in 2001 and professor in 2009.

Two neuroscientists were awarded prestigious NARSAD Independent Investigator grant by the New York City–based Brain and Behavior Research Foundation (BBRF). Adam Kepecs and Bo Li, both associate professors, were among 40 mid-career scientists from 30 institutions in 16 countries selected for the honor this year. The awardees were selected by the Foundation’s Scientific Council, comprised of 150 leading experts across disciplines in brain and behavior research, including two Nobel Prize winners and four former directors of the National Institute of Mental Health.

Adam will take advantage of new light-based genetic tools to monitor and manipulate the activity of cells that release the neurotransmitter serotonin in mice. Serotonin levels are modulated by drugs to treat depression, anxiety, panic disorder, chronic pain, and other psychiatric conditions, but when serotonin neurons are activated during behavior remains unclear. His experiments are designed to clarify the behavioral roles of these central serotonergic neurons.

Bo aims to elucidate the mechanisms that cause abnormal fear processing in anxiety disorders such as post-traumatic stress disorder. He will use his grant to investigate brain circuits in the anterior insular cortex and the amygdala, brain regions that are activated during the experience of fear and hyperactive in people with anxiety disorders.

The American Association for Cancer Research (AACR) honored Assistant Professor Christopher R. Vakoc, M.D., Ph.D., with the 35th annual AACR Outstanding Achievement in Cancer Research Award. Since 1979, the AACR Award for Outstanding Achievement in Cancer Research has been awarded to an investigator younger than 40 years of age to recognize his or her meritorious achievements within the field of cancer research.

Chris was recognized for his groundbreaking discoveries in the areas of epigenetics and cancer biology. His research on the basic molecular mechanisms that control leukemias have revealed an essential connection between epigenetic regulation and oncogenesis. This work has led to the development of potential new therapeutic approaches—including a drug called a bromodomain inhibitor—currently being evaluated in early-stage clinical trials.

Associate Professor Michael Schatz received a 2015 Alfred P. Sloan Foundation Research Fellowship. Mike was one of 126 outstanding early-career scientists from the United States and Canada recognized by the Foundation this year. Past honorees include the famous physicist Richard Feynman and 42 others who went on to win the Nobel Prize.

Mike is a quantitative biologist and a faculty member of CSHL’s Simons Center for Quantitative Biology. From the very start of his career he has been recognized as an innovator in cloud computing and “big data.” At CSHL, Mike has applied his quantitative insights to diverse problems, ranging from methods of assembling plant and animal genomes from raw DNA sequencing data to the analysis of large data sets generated in studies of people with diseases such as cancer and autism.

Dean of the Watson School of Biological Sciences Alexander Gann was awarded a 2015 Guggenheim Fellowship in science writing. Alex will use the award to write a book about the hunt for the so-called homeotic (or Hox) genes—the genes that control how the bodies of all animals are shaped. “Mutants in these genes were first identified, in the
fruit fly *Drosophila melanogaster*, 100 years ago,” Gann says. “They were striking for their gothic, fairytale-like appearance: legs growing out of heads in place of antennae, or an extra sets of wings growing out of the body.” The genes were isolated in 1982 and found to be conserved—shared across evolutionary time—in all animals. This result heralded in the era of molecular developmental biology and the now-flourishing field of Evo-devo. Alex intends to chronicle both the fascinating science and the rich menagerie of characters behind the discovery.

Guggenheim Fellowships are awarded to individuals who have established themselves as exceptional scholars and artists. James Watson, chancellor emeritus, received two Guggenheim Fellowships, his first, in 1965, for the writing of the original edition of *The Double Helix*. Former CSHL scientists honored as Guggenheim scholars include Barbara McClintock (1933), Salvador Luria (1942), Renato Dulbecco (1957), and Rich Roberts (1978), as well as the current head of Simons Center for Quantitative Biology, Adam Siepel.

Newly appointed CSHL Fellow Jason Sheltzer received the 2015 Early Independence Award from the National Institutes of Health (NIH) High Risk, High Reward Research Program. With a Ph.D. in Biology from MIT, Jason joined CSHL in August to pursue cancer research. He was also named one of *Forbes* magazine’s “30 under 30 in Science” earlier in the year. Jason is studying genes that may make a difference between tumors that are benign and those that are cancerous and likely to spread to other parts of the body, a process called metastasis. “In order to uncover new genes that play a role in cancer progression and to increase our understanding of the molecular differences between fatal and nonfatal tumors, we are analyzing data from cancer patient survival studies,” explains Jason. He will use the latest genome-editing technology, called CRISPR, to establish the molecular links between these genes and cancer prognosis.

Luis A. Mejia, Ph.D., a postdoctoral investigator, was named a 2015 Arnold O. Beckman Postdoctoral Fellow. Mejia works in the laboratory of Bo Li and came to CSHL following doctoral training at Harvard University, where he was awarded an NIH NRSA predoctoral fellowship for his research in neuroscience. His project is of fundamental importance to understanding behavioral flexibility and has the potential to yield significant insights into the basis of human neuropsychiatric disorders. Mejia aims to understand how neural circuits encode behavioral flexibility and how changes in neural activity can give rise to maladaptive, abnormally repetitive behaviors. Such behaviors are seen in illnesses such as obsessive–compulsive disorder, autism spectrum disorder, Tourette’s syndrome, attention-deficit hyperactivity disorder, and anxiety disorders. Mejia is establishing a mouse model of obsessive behavior that will enable him to evaluate the role of specific neuronal populations in these behaviors. He will focus on the striatum, a part of the brain important for behavioral flexibility.

Ian Peikon is the fourth CSHL graduate student to have won the prestigious (2015) Harold M. Weintraub Graduate Student Award. In April, Peikon graduated from the Watson School, where his studies focused on developing innovative and affordable methods to map the connections between neurons in the brain. A Long Island native who went to Bethpage High School, Peikon came to CSHL in 2009 with a BSE in Biomedical Engineering from Duke University.

**Promotions and New Hires**

Congratulations to the faculty who were promoted to Associate Professor: Florin Albeanu, Gurinder Atwal,
Alexander Krasnitz, Stephen Shea, and Christopher Vakoc. Camila dos Santos was named Assistant Professor.

The Laboratory also welcomed CSHL Fellow Jason Sheltzer. Charles S. Ryan joined as Vice President, General Counsel.

**Education Programs**

**Banbury Conference Center**
The Laboratory’s think tank for biology and science-and-society issues attracted more than 600 participants this year. One highlight occurred in March, when those attending the “Exercise Science and Health” meeting attempted a critical review of the data and examined how far they can be trusted. As is often the case, it was difficult to get members of different fields with different practices to reach a consensus.

“HIV-1 and How to Kill a Killer” was the first meeting on HIV and AIDS to be held at Banbury since the early 1990s. Five participants in those early meetings were here in 2015. They and other attendees examined the vexed issue of where the virus hides in “cured” patients until it reappears years later. The meeting on “Therapeutic Use of Ketamine for Treating Severe Depression: Risks and Potential” was a follow-up to a meeting held in 2013 on the biology and pharmacology of an anesthetic drug being used with notable success in depression that does not respond to other treatments and as a suicide preventive. This year’s meeting dealt with the question: Why, if ketamine is so effective in treating severe depression, is it not widely used? The answers involve complicated social, economic, and regulatory issues.

**DNA Learning Center**
In 2015 the DNALC served 20,570 students who attended field trips at facilities in Cold Spring Harbor as well as DNALC West and the Harlem DNA Lab. An additional 8908 students completed labs in their own schools, led by DNALC staff, whereas 1348 students attended week-long summer camps. The Center’s popular Urban Barcode Project had a successful fourth year, with students presenting research posters and giving oral presentations at the American Museum of Natural History. Student teams supported by teachers from 20 schools in Nassau, Suffolk, and Queens counties presented project results at the inaugural Barcode Long Island symposium in June.

This year, the DNALC concluded negotiations to develop a DNA Learning Center Asia in Suzhou Industrial Park (SIP), near Shanghai, China. DNALC Asia has been registered as an “internal” Chinese NGO, sponsored by CSH Asia Conferences. It is being developed on a 270,000-square-foot site, and its first set of teaching labs will have twice the capacity of the Dolan DNALC facility in Cold Spring Harbor Village.

The Dolan DNALC facility is now the home of a precise life-size reproduction of “Ötzi the Iceman,” the mumified remains of a European male who lived about 5300 years ago. The unique replica provides the entry point for exploration of human origins, a longtime interest of the center. The exhibit is designed to complement experiments pioneered by DNALC, in which students use their own DNA as an entrée to the study of the human family and its dispersion across the planet.

Ohio high school senior Ryan Chester was awarded the first-ever Breakthrough Junior Challenge prize during a live broadcast hosted by Seth MacFarlane on the National Geographic Channel. The prize included a
state-of-the-art science and mathematics laboratory designed by the DNALC. Ryan’s high school science lab was last renovated during the 1950s. The new lab will bring the school’s classroom to 21st-century standards with a design based on the DNALC’s highly successful open, collaborative laboratory concept.

**Cold Spring Harbor Laboratory Press**
The Press publishes eight journals, has 200 books in print and electronic form, and two web services. In 2015, a new journal joined the list. Subtitled “a journal of precision medicine” *Cold Spring Harbor Molecular Case Studies* illuminates the traditional medical case report with advanced laboratory investigations such as genome sequencing.

Confidence in this new publication is sustained by the success and reputation of the other Press journals. Online, the Press journals had a record download of more than 13.4 million full-text articles.

The year saw increasing adoption of bioRxiv, the online distribution service for preprints of research papers in the life sciences, founded in 2013. It permits scientists to make their work immediately available to the research community and receive feedback on draft manuscripts before submitting them to journals. The monthly rate of manuscript submission doubled between May and December and represented more than 1500 institutions in 40 countries. Launched with seed funding from the Laboratory, bioRxiv has since received critical support from The Lourie Foundation. Its emergence demonstrates that the Laboratory is continuing to pioneer new ways of advancing science by sharing results and ideas.

**Watson School of Biological Sciences**
In 2015 the Watson School graduated its 12th class. Students continued to graduate considerably faster than students in comparable Ph.D.-granting institutions and demonstrated an ability to secure excellent jobs. Twenty-one graduates have secured tenure track faculty positions. Seven have already been promoted to Associate Professor.

At the graduation ceremony, 12 students were awarded Ph.D. degrees, bringing the total since the school’s inception to 83. During the year, scientific papers published by students of the school appeared in major journals, bringing the cumulative total to more than 300.

At the ceremony, Honorary Degrees were presented to Charlie Rose, Lord David Sainsbury, and Dr. Hanna Gray, as part of the celebration of the 125th anniversary of CSHL.

A leader in education and an advocate for scientific research, Hanna Holborn Gray, Ph.D. served as president of the University of Chicago from 1978 to 1993 and was the first female president of a major university in the United States. Lord David Sainsbury was Minister for Science and Innovation in the United Kingdom from 1998 and 2006. He established the Gatsby Charitable Foundation in 1967 with a particular emphasis on plant science and neuroscience. Journalist Charlie Rose was honored as a leader in public engagement, insight, and inquiry into contemporary topics of science and society.

In August, the WSBS matriculated seven new students, selected from among 310 applicants and representing the United States, Lithuania, Russia, the United Kingdom, and Vietnam. Other new graduate students entered as visitors from other institutions, including seven from Long...
Island’s Stony Brook University; other current visitors hail from institutions including Cornell University and the National Centre for Biological Sciences, India.

**Meetings & Courses Program**

In 2015, scientific meetings attracted almost 7600 participants to the main campus. When combined with participants in the China meetings, the program drew about 10,500 scientists in all. The year saw the continuation of many successful annual and biennial meetings, as well as the introduction of several new meetings, focusing on Immunology, Probabilistic Modeling in Genomics, and Genome Engineering. The latter meeting was a 2015 highlight, featuring the CRISPR genome editing technique, attracting more than 400 participants from around the globe.

In June, Cold Spring Harbor Asia Conferences hosted a special meeting in Suzhou, bringing together stakeholders in many of the national brain projects recently initiated around the world.

More than 750 instructors, lecturers, and assistants came to teach at CSHL from universities, medical schools, research institutes, and companies around the world. During the year, about 700 trainees—who included advanced graduate students, postdocs, and faculty—attended courses lasting from one to three weeks.

In 2015 renewal was secured for course funding from the Howard Hughes Medical Institute, a long-time benefactor. New major multiyear grants were received from the Helmsley Charitable Trust and the National Institute of General Medical Sciences.

**Infrastructure**

The Laboratory completed two major construction projects during the year: a 10,000 square foot extension was added to the Woodbury Genome Center to house a new Preclinical Experimental Therapeutics Facility (PETx), and the Nicholls Biondi Hall was erected as a venue for scientific
poster sessions of the Meetings & Courses Program. With these two major projects completed, the Laboratory turned to a number of smaller projects to renovate and upgrade facilities and mechanical systems.

Library

The CSHL Library and Archives continued its commitment to exploring and preserving the history of molecular biology while also looking to the future to improve scientific communication to students, scientific investigators, and the public. An example of this outreach is the Cold Spring Harbor High School Eugenics Presentation, given again this year. Over the past four years, more than 100 high school seniors have come to CSHL for a tour, presentation, and discussion of eugenics as part of the local public school’s History of Science class.

“A Heroic Voyage: Sydney Brenner’s Life in Science” symposium and exhibition in October in Singapore was organized by Mila Pollock and A*Star, the Singapore Agency for Science, Technology, and Research. This tribute celebrated the Nobel Laureate, emphasizing his many scientific accomplishments and his leading role in the development of biomedical research in Singapore. The exhibit was curated by the CSHL Library and Archives, featuring photographs and memorabilia from our Brenner collection. We are grateful for Brenner’s donation of his scientific papers to the CSHL Archives and for the Sydney Brenner Research Scholarship in the History of Molecular Biology, Genetics, and Biotechnology. The 2014–2015 scholarship recipient was Erika Langer, a doctoral candidate at the University of California, San Francisco, studying the early years of the CSHL yeast group.

“The Evolution of Sequencing Technology: A Half-Century of Progress” was organized by Mark Adams, Nigel Brown, Mila Pollock, and Robert Waterston from July 16 to 19, as part of the Genentech Center for the History of Molecular Biology and Biotechnology program. Prominent researchers participating in DNA sequencing from its beginnings were brought together to describe how the technology developed, its effect on the biological sciences, and its role in current and future research.

Other highlights of 2015 included:

• Hermann Muller Exhibition, February
• Exhibit and website honoring the Albert Lasker Basic Medical Research Award to Evelyn Witkin for discoveries regarding the DNA-damage response
Highlights of the Year

- James D. Watson lecture “Celebrating 50 years of Molecular Biology of the Gene”
- Women in Science Exhibition, October

Trustees

George D. Yancopoulos, M.D., Ph.D., Chief Scientific Officer of Regeneron Pharmaceuticals, Inc. and President of Regeneron Laboratories was elected to the Board of Trustees.

Dr. Yancopoulos joined Regeneron in 1989 as its Scientific Founder and is currently Chief Scientific Officer and President of Regeneron Laboratories. Together with key members of his team, he is a principal inventor and developer of Regeneron’s four FDA-approved drugs—Praluent (alirocumab) Injection, Eylea (aflibercept) Injection, Zaltrap (ziv-aflibercept) Injection for Intravenous Infusion, and Arcalyst (rilonacept) Injection—as well as of Regeneron’s foundational technologies for target and drug development, such as its proprietary TRAP technology, VelociGene and VelocImmune.

In the 1990s, Dr. Yancopoulos was the 11th most highly cited scientist in the world, and in 2004 he was elected to both the National Academy of Sciences and the American Academy of Sciences. Dr. Yancopoulos has long-term ties to the CSHL. Together with his Ph.D. mentor, Dr. Frederick W. Alt, who now heads the CSHL Scientific Advisory Board, he taught the CSHL cloning course for many summers in the 1980s.

Development

More than $4.5 million was raised at the 10th Double Helix Medals dinner (DHMD) in November. The gala was held for the first time at the American Museum of Natural History (AMNH) in New York City and honored Katie Couric, Anne Wojcicki, and David Botstein, champions of research to benefit mankind.

The 14th annual Women’s Partnership for Science luncheon was held in September, featuring guest speakers Anja Hohmann and Maria Nattestad, Watson School Ph.D. students. Since 2002, the event has raised $1.5 million for pioneering research. This year’s event included a special honor for enthusiastic Laboratory supporter, Cathy Soref. Cathy has been part of the CSHL community for more than 25 years. In 2006, Cathy initiated the Double Helix Medals dinner.
CSHL’s 22nd annual golf tournament raised $230,000 for research and education honoring Trudy and Tom Calabrese—former CSHL Association President and founding CSHL Corporate Advisory Board member, respectively.

Cold Spring Harbor Laboratory (CSHL) was among 17 Long Island–based organizations to receive funding from the 2015 Long Island 2 Day Walk to Fight Breast Cancer. The $19,000 donation received by CSHL will support breast cancer research in the laboratory of Professor Alea Mills, Ph.D. In addition to its participation in the events, CSHL heads The LI2DAY Walk Scholarship Committee, which awarded six $2500 scholarships to Long Island high school seniors who have a parent or guardian with breast or other women’s cancer.

A special thank you this year to Chancellor Emeritus James D. Watson and his wife Liz, who donated $1 million to the Laboratory in support of biomedical research and education programs. To date, Jim and Liz have donated more than $5 million to the Lab in addition to many other invaluable contributions, ensuring the Lab’s leadership in contemporary biology and genetics research and education.

The Watson gift includes an endowment to support a special annual scientific meeting called “Double Helix Day,” in memory of February 28, 1953—the day that Watson and his colleague Francis Crick discovered the double helix structure of DNA.

**Community Outreach**

**CSHL Public Lectures—Special 125th Anniversary Series**

May 3—Silvia Earle, Ph.D., National Geographic Explorer in Residence; *Saving Our Oceans.*

June 17—Michael Wigler, Ph.D., Professor, Cold Spring Harbor Laboratory; *Seeking Out Cancer*; co-sponsored by CSHL, US Trust—Bank of America, North Shore-LIJ and St. Johnland Nursing Center.
October 4 — Harold Varmus, M.D., Weill Cornell Medical College; Nobel Laureate; Former Director, National Cancer Institute; *Biomedical Research: Then and Now.*

October 13 — Peter Neufeld, Co-founder and Co-director of the Innocence Project; *How the Lessons of DNA Transform Criminal Justice*, 2015 Lorraine Grace lectureship on societal issues of biomedical research.

October 29 — David Spector, Ph.D., Professor and Director of Research, Cold Spring Harbor Laboratory; *Searching for New Ways to Halt the Progression of Breast Cancer*; hosted by Mather Hospital as part of Paint Port Pink events in Port Jefferson, New York.

November 8 — David Tuveson, M.D., Ph.D., CSHL Professor, Lustgarten Foundation Director of Research; Craig Devoe, M.D., Chief, Hematology and Medical Oncology, The Monter Care Center, North Shore-LIJ; *A Partnership to Defeat Pancreatic Cancer: CSHL & The Lustgarten Foundation.*

November 12 — Stephen Shea, Ph.D., Associate Professor, Cold Spring Harbor Laboratory; *Cocktails & Chromosomes* Inaugural event at Finley’s of Greene Street in Huntington, New York.

November 16 — Neil Shubin, Ph.D., Robert R. Bensley Professor, Organismal Biology and Anatomy, University of Chicago; *Your Inner Fish.*

**CSHL Public Concerts—2015**

March 27 — Martin Kasik, piano
April 17 — Jiayin Shen and Alan Woo, piano duo
May 1 — Julia Bullock, soprano (with piano)
May 15 — Trio Solisti, violin, cello, and piano
August 21 — Southampton Arts Festival Chamber Orchestra
September 11 — In Mo Yang, violin (with piano)
September 18 — Ko-Eun Yi, piano
October 2 — Mei Rui, piano

**Looking Forward**

Thank you to our faculty, students, and staff for their hard work this anniversary year. History offers us pride and inspiration as we all look forward to continuing CSHL’s leadership role in molecular biology and genetics research and education.

Bruce Stillman, Ph.D., F.R.S.
President and Chief Executive Officer
CHIEF OPERATING OFFICER’S REPORT

As Bruce Stillman rightly points out in his President’s Report, basic discovery science, the heart of Cold Spring Harbor Laboratory’s mission, is becoming increasingly expensive. This is a result of many factors, including escalation in expenses associated with scientific equipment, supplies, and recruitment and retention of the world’s best scientists. Unfortunately, this upward expense spiral has coincided with a 20% inflation-adjusted decline in the budget of the National Institutes of Health over the last decade.

CSHL has navigated its way through this challenging environment by excelling in a few critical areas. One of those is the success of our faculty in obtaining federal grants. Over the last year, their success rate was 46% as compared to the national average of 17%. This is simply a reflection of the excellence of our research and the vigor with which our investigators, in partnership with our Office of Sponsored Programs, write and submit high-quality federal grant applications. In other words, we continue to obtain a bigger slice of a shrinking pie.

At the same time, the Laboratory has been very successful in attracting funds from private foundations and philanthropy. One need only look at the shift in the mix of our research funding over the last decade. In 2005, federal grants made up nearly 60% of the total, with private sources at 20%. Ten years later in 2015, federal grants constitute 44%, with private sources at 29%. It is important also to note that the remaining 27% of the research budget is now funded by Laboratory sources, including the annual fund and spending from our endowment funds, both of which are reflective of a highly energetic and successful fundraising effort. For this we have to thank our Development Office, our Board of Trustees, and the many devoted supporters of CSHL.

The Laboratory’s endowment fund, in some respects its lifeblood, has grown impressively from $215 million in 2008 to $450 million at year-end 2015. This growth is the result of many generous endowment gifts in addition to investment returns on the funds. Since the 2008 financial crisis, we have benefitted from strong financial markets and a sound investment strategy developed by the Investment Committee of our Board of Trustees. While in calendar year 2015 CSHL investment returns were flat, we have not had a down year in the last seven and in four of those years we achieved double-digit returns. This combination of fundraising and positive investment returns is inarguably good news. However, as stock markets hover near all-time highs and interest rates remain stubbornly low, it is increasingly difficult to plan for investment returns on the endowments that are substantially in excess of our spending rate. This is a challenge now being addressed.

So we are doing many things well at the Laboratory and, as a result, are on much firmer financial footing than many of our peer institutions. However, there is a big-picture concern over which we have less control. This problem was well articulated by honorary degree recipient Senator Tom Harkin, who delivered the commencement address at the 2016 Watson School graduation ceremony. Citing the decline in federal research funding, the senator expressed deep concern over the antiscience discourse in the United States. In his words, “I think there is a clear and present danger that the U.S. will lose its leadership in the years ahead. This will have a devastating impact across our economy with lower growth and fewer innovations. We must put a stop to the growth of the tumor of anti-intellectualism and antiscience in our body politic.”

Strong words for sure, but a warning worth heeding. If we become a society where dogma and beliefs supplant empirical evidence and facts, it will be difficult even for institutions as fine as Cold Spring Harbor Laboratory to thrive.

W. Dillaway Ayres, Jr.
Chief Operating Officer
Long-Term Service

The following employees celebrated milestone anniversaries in 2015:

45 years  Madeline Wisnewski
35 years  Linda Rodgers
30 years  Lisa Manche, David Spector
25 years  Edward Campodonico, Clare Clark, Beicong Ma, George Newell, Barbara Purcell, Ronald Romani, Hock Yew Teo, Nicholas Tonks
20 years  William Bishop, Barry Burbach, Giuditta Carino, Kenneth Chang, Jodi Coblentz, Joshua Dubnau, Leemor Joshua-Tor, Alyson Kass-Eisler, Mary Muno, Mona Spector, Benjamin Veneable

Front row (left to right): Leemor Joshua-Tor, Lisa Manche, Madeline Wisnewski, James Watson, Beicong Ma, Jodi Coblentz, Alyson Kass-Eisler, Linda Rodgers; back row (left to right): William Bishop, Barry Burbach, Ronald Romani, Benjamin Veneable, Bruce Stillman, David Spector, Hock Yew Teo, George Newell, Dill Ayres.
15 years Paula Abisignio, Carol Brower, Amy Burrows, Elna Carrasco, James Decker, Wesley Dreusike, Jeffrey DuPree, Suzanne Esposito, Leo Greene, Matthew Hoyer, Z. Josh Huang, Charles Prizzi, Denise Roberts, Michael Ronemus, Mary Sabala, John Schindler, Yi-Jun Sheu, Jill Stone, Peter Van Buren, Xiaoqun Zhang, Steven Zielinski
CANCER: GENE REGULATION AND CELL PROLIFERATION

Camila dos Santos’ laboratory studies the epigenetic regulation of normal and malignant mammary gland development, with an emphasis on the alterations brought by pregnancy. Significant changes mark the pre- and postpubescence mammary developmental stages, but those associated with pregnancy have the greatest effect on cellular function, tissue reorganization, and breast cancer susceptibility. Her group has recently found that mammary glands react differently to a second pregnancy than they do to the first one, with associated changes in DNA methylation. These findings suggested that pregnancy changes the state of mammary cells, and these may permanently alter how they react to the next pregnancy. In addition, the dos Santos lab is exploring how the pregnancy-induced epigenetic changes might influence cell transformation and the risk of breast cancer. This research utilizes genomic and computational approaches to define the pre- and post-pregnancy mammary epigenome. An additional objective of the dos Santos laboratory is to use functional genomics to discover novel transcriptional regulators that modulate mammary stem cell self-renewal, lineage specification, and cell transformation. The long-term objective of Camila’s group is to improve the notion of the mammary epigenome during normal development and use this information to gain insight into new preventive and curative strategies to target breast cancer.

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 HPV 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. Current research focuses on the mechanism that initiates the entire process of DNA replication in eukaryotic cells. At the heart of this mechanism is a protein that binds to “start” sites on the chromosomes, called the origin recognition complex, ORC. The Stillman lab is part of an ongoing 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. These images offer molecular insights into how the helicase is loaded onto DNA. Stillman’s research also focuses on the process by which duplicated chromosomes are segregated during mitosis. They found ORC at centrosomes and centromeres, structures that orchestrate chromosome separation during mitosis. At centromeres, ORC subunits monitor the attachment of duplicated chromosomes to the mitotic spindle that pulls the chromosomes apart when they are correctly aligned. Stillman’s team has discovered that mutations in the Orc1 protein 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 people with extreme dwarfism and small brain size, but normal intelligence.

Cancer can be understood as a disease of dysfunctional gene expression control. Research in Chris Vakoc’s lab investigates how transcription factors and chromatin regulators cooperate to control gene expression and maintain the cancer cell state. This work makes extensive use of genetic screens to reveal cancer-specific functions for transcriptional regulators, as well as genomic and biochemical approaches to identify molecular mechanisms. One theme that has emerged from their efforts is that blood cancers are often vulnerable to targeting transcriptional coactivators, such as BRD4 and the SWI/SNF chromatin-remodeling complex. Vakoc’s team demonstrated that chemical inhibition of BRD4 exhibits therapeutic effects in mouse models of leukemia, a finding that has motivated ongoing clinical trials in human leukemia patients. The Vakoc lab has also developed a CRISPR-Cas9 screening approach that can reveal individual protein domains that sustain cancer cells. Their lab is now deploying this technology in a diverse array of human cancers to reveal therapeutic opportunities and basic mechanisms of cancer gene control.
The dos Santos laboratory focuses on the investigation of normal and malignant mammary gland development. In particular, we are interested in how epigenetic factors and modifications control mammary gland stem cell self-renewal, cell differentiation, and malignant transformation. We currently have three main projects in the lab.

Epigenetic Reprogramming of Breast Cancer Risk
W. Frey, R. Khatiwala, C. dos Santos

There is an epigenomic signature specific to postpregnancy mammary epithelial cells. This pregnancy-induced epigenome influences the regulation of gene expression and may be involved in pregnancy-induced breast cancer protection, one of the strongest factors that reduce the risk of breast cancer development in humans and rodents. Our goal is to identify the factors that influence the resistance to breast cancer.

Our experimental design is to isolate mammary epithelial cells from female mice that are predisposed to develop cancer. In these mouse lines, the human oncogene C-MYC is under a doxycycline-inducible system. To date, we have isolated mammary stem cells from female mice exposed to pregnancy hormones and from control female mice (no pregnancy hormone exposure) and tested their capacity to undergo malignant transformation in vivo. We learned that transgenic mammary epithelial cells harvested from female mice exposed to pregnancy hormones fail to proliferate on the induction of oncogene expression. We have isolated RNA from these cells and found deregulation of genes with a role in cancer progression. These same genes are up-regulated on mammary epithelial cells from virgin transgenic mice.

Our future goal is to use CRISPR–CAS9 genomic editing to abolish or overexpress these differentially expressed genes and to evaluate the effects of their manipulation on proliferation, apoptosis, and malignant transformation.

Mimicking the Protective Effects of Pregnancy with Pimozide
P. Ferreira Papa, C. dos Santos [in collaboration with G. Hannon, CSHL]

Among women, breast cancer represents the most common cause of cancer death, with an impressive number of 1.7 million new cases diagnosed in 2012 worldwide. Various treatments for this cancer have emerged over the past decades; however, preventive therapies are poorly investigated. Studies have shown that at an early age of full pregnancy, the long-term risk of developing breast cancer in humans is reduced by >30%. Our previous work has shown that pregnancy stably changes the DNA methylation status of mammary epithelial cells. Some of those modifications map to regions bound by the transcription factor STAT5. Curiously, these long-lasting effects of the first pregnancy also elicit a rapid development of the mammary gland in a second pregnancy.

Now, our goal is to characterize new preventive strategies that mimic the effects of pregnancy on breast cancer risk. Pimozide (Orap or PIM), a U.S. Food and Drug Administration (FDA)-approved drug, has been previously reported to increase the levels of prolactin and induce lactation in humans. Additionally, PIM has been suggested to attenuate STAT5 activity to the same levels found in postpregnancy mammary epithelial cells. Therefore, PIM treatment could mimic some of the pregnancy-related signals and may influence breast cancer development.

To date, we have treated nulliparous (virgin) and parous (postpregnancy) female mice with PIM to investigate its effects on mammary gland development. Our preliminary results show a significant increase
in the mammary gland branching morphogenesis in mice treated with PIM, suggesting a potential effect of PIM in a pregnancy-like development of the mammary gland. We are now analyzing the transcriptome and the epigenome of mammary epithelial cells exposed to further understand the effects of PIM on global gene regulation.

Additionally, we are investigating the effects of PIM treatment on the development of mammary tumors. Here, we are using chemically induced (DMBA treatment) and genetically predisposed (BRCA1MT and MMTV-PyMT) mouse models of mammary tumors. The hope is to find a decrease in the frequency of mouse mammary tumors in these models and perhaps a suitable strategy to prevent breast cancer development in humans.

**Defining Breast Cancer Epigenetic Dependencies**

A. Chaudhry, C. dos Santos

The goal of our project is to search for epigenetic factors with unique roles in mammary stem cell maintenance and cell differentiation. We compared gene expression and DNA methylation of the major mammary epithelial cell types and found many novel transcriptional regulators preferentially expressed by mammary stem cells (MaSCs) and luminal progenitor cells. Among the many targets, we focus on the characterization of BPTF, a largely unstudied regulatory factor and core subunit of the NURF chromatin-remodeling complex, as a potential regulator of MaSCs. We engineered a conditional mouse strain to specifically inactivate Bptf in MaSCs (KRT5\textsuperscript{CRE-ERT2BPFTfl/fl}), and found that MaSC development is mostly dependent on Bptf function. However, Bptf ablation at postpubescent stage (10 wk or older) did not affect mammary gland homeostasis, suggesting that Bptf may play an important role in controlling the growth kinetics of MaSCs at specific mammary developmental stages.

The notion that Bptf inhibition has a greater impact on rapidly expanding MaSCs during early development led us to investigate the potential role of Bptf in cancer development. In fact, genomic analyses suggest that \textit{BPTF} messenger RNA (mRNA) up-regulation is associated with poor prognosis of human breast invasive carcinomas. Our analysis additionally showed an increase in BPTF protein levels in human breast cancer tissue as compared with almost undetectable levels in normal epithelial tissue. We also investigated the effect of BPTF short hairpin RNA (shRNA) knockdown in several cancer cell lines, and found that BPTF inhibition specifically blocked growth of human breast cancer cell lines, with no effect on the growth of lung, ovarian, and melanoma human cell lines. This suggests that BPTF may represent a breast-specific factor and its inhibition a promising therapeutic approach to target breast cancer. It is worth mentioning that BPTF contains a bromodomain and therefore represents a "druggable" factor. In fact, we have recently coauthored a manuscript describing the first BPTF bromodomain inhibitor.

We hypothesize that BPTF, via its chromatin remodeling function, controls an essential regulatory network that supports cell proliferation, cell cycle progression, and differentiation, and the effects of its inhibition may represent an ideal sweet spot to target breast cancer.

**PUBLICATION**

Human development requires the regulated expression of thousands of genes in hundreds of distinct cell types throughout life. One requirement for this process is that each cell must contain an intact, functional genome. Furthermore, there is a set of rules for how the genes in our genomes coordinate their activities, and these rules are encoded within gene regulatory networks. Many human diseases occur when these basic processes are altered—either through altering the genome itself (as in the mutations seen in cancers) or through altering the way in which genes interact within gene networks. The focus of our lab is to understand how mutations in our genomes lead to both alterations in the function of the mutated gene itself and the repercussion of these alterations on the hundreds of other neighboring genes within the network. To this end, the Hammell 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 like cancer take advantage of the cell’s ability to rewire its own regulatory networks into programs that serve the needs of the cancer cells.

Much of this work involves tight collaborations with other experimental groups at CSHL. One collaboration, with Josh Dubnau’s lab, is exploring the contribution of endogenously encoded retroviral-like elements in human genomes to neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Another example involves a collaboration with Marja Timmermans’ lab to understand the diversity of small RNA regulators that control both retroviral-like elements in the maize genome and genes that dictate leaf patterning in maize development. 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 regulation of gene expression controls cellular function.

**Endogenous Retroviral-Like Elements May Contribute to Neurodegeneration**

Y. Jin, O. Tam, N. Rozhkov [in collaboration with J. Dubnau, CSHL]

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 amyotrophic lateral sclerosis (ALS), frontotemporal lobar degeneration (FTLD), and Alzheimer’s disease (AD). 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. In collaboration with the Dubnau lab, this group has explored the novel hypothesis that TDP-43 normally plays a large and hitherto uncharacterized role in regulating the expression of transposable elements (TEs). TEs are retroviral-like elements encoded within our genomes 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 hTDP-43 protein. Members
of the Hammell lab 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, a disease characterized by TDP-43 proteotoxicity. Although these studies support a role for TDP-43 in regulating TE expression, our future goals are centered on determining the role for misregulation of TEs in neurodegenerative disease. Ongoing research in the lab is focused on three main areas: (1) improving our ability to detect active transposons in ALS and FTLD patient samples, (2) examining the degree to which transposons and other retroviral-like elements are interfering with cellular function in ALS and FTLD patient tissues, and (3) understanding the basic biology of how TDP-43 interacts with the general transposon control machinery.

Transposable elements have been historically difficult to study because of their highly repetitive nature. Nearly half of the human genome is composed of TE-derived sequences, with millions of copies of TEs scattered throughout the chromosomes. Although most of these copies are nonfunctional, thousands of TEs retain the ability to mobilize and create new copies of themselves elsewhere in the genome. The difficulty lies in differentiating these active TEs from the millions of other harmless copies with nearly identical sequences. This presents both a technical challenge for experimentally isolating TE-derived sequences from the genomes of cells and a computational challenge for determining where each TE copy originates in the genome of a particular sample. Members of the lab have recently developed novel statistical inference methods to solve the computational challenge of analyzing TE expression in sequencing studies (Jin et al. 2015). These statistical inference methods have been used to examine the basic mechanisms of controlling TE expression and activity in germline tissues in collaborative studies (Goh et al. 2015; Wasik et al. 2015). Ongoing efforts will establish optimized analysis protocols for many different types of TE studies (genome resequencing studies, chromatin association studies, etc.). In addition, several members of the lab are developing optimized protocols for isolating and identifying novel TE insertion sites in the genomes of individual cells, in collaboration with the Dubnau lab. Together, these efforts will provide the tools with which to determine the extent of TE activity in patient samples.

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 and the well-characterized plant model Arabidopsis. 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, this 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. Analysis of maize tasiRNAs, a group of mobile endogenous 21 nt 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-nt 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 miRNAs from Arabidopsis are conserved in maize, leaving a wealth of novel miRNAs to explore. Results from these studies were recently published in two manuscripts (Petsch et al. 2015). Further exploration of maize small RNA pathways will explore the novel maize microRNAs and how the expression and activity of these small RNAs vary within maize populations and across related grasses. Our lab has developed novel algorithms for the identification of phased small RNAs, for microRNA gene identification, and for microRNA target interaction analysis. These algorithms are being applied to deep sequencing data sets of small RNAs and mRNAs.
isolated from developing maize tissues in several inbred maize populations.

Mechanisms of Acquired Drug Resistance in Melanoma
A. Patel, Y.-J. Ho

The genetic basis of melanoma development is fairly well understood, with activating mutations in the oncogene BRAF occurring in a majority of melanoma patients. Specific inhibitors that target activated BRAF as well as the downstream MAPK/ERK signaling pathway have been developed that dramatically reduce the growth of melanoma cells in patients. However, the effects of these drugs typically extend patient life span for 6 months or less, as the tumors rapidly develop resistance to these targeted therapies. Although some tumors resistant to BRAF inhibitors acquire additional genetic lesions that elevate ERK or AKT signaling, an astounding number of therapy-resistant cell lines establish resistance without significant alteration of the genome. Members of the Hammell lab are developing computational algorithms to understand what changes take place in melanoma cells that develop resistance to BRAF inhibitor therapy and how this changes based on the genotype of different melanoma tumor types.

One goal of the lab is to classify melanoma tumors by both their sets of cancer-specific mutations (their genomic subtype) and the gene transcripts they express (their transcriptional subtype). Large-scale sequencing surveys of melanoma patients have suggested that hundreds of genomic subtypes exist, such that each patient has their own distinct mutational profile, with a few commonly mutated genes alongside thousands of patient-specific mutations. However, using novel algorithms to analyze the sets of gene transcripts expressed by these tumors, we have determined that cutaneous melanoma can be largely classified into a small number of transcriptional subtypes, and that these transcriptional subtypes are highly predictive of overall patient survival rates. Moreover, tumors with each distinct subtype can be seen in human patient samples, in mouse xenograft models of melanoma, and in cell culture systems. This allows for the subtype-specific modeling of melanoma progression in animal and cell culture systems. Ongoing research is using cell culture models of each melanoma subtype to determine whether response to therapy differs for melanoma cells of different subtypes, and whether this can inform the likelihood of developing therapeutic resistance through different cellular pathways.

PUBLICATIONS
We study the molecular basis of nucleic acid regulatory processes, RNAi and DNA replication in particular. We use the tools of structural biology, biochemistry, and biophysics to study proteins and protein complexes associated with these processes to elucidate how they work. X-ray crystallography and other structural techniques enable 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 and Noncoding RNAs

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. To get a true mechanistic understanding of these pathways, however, we must understand how the components of the RNAi machinery work at a molecular level.

The let-7 Regulatory Network

Although many studies focus on the identification of microRNA (miRNA) targets and the various downstream mechanisms of gene silencing, we are interested in the regulation of a particular miRNA, let-7. The pluripotency factor Lin28 inhibits the biogenesis of the let-7 family of mammalian microRNAs. Lin28 is highly expressed in embryonic stem cells and has a fundamental role in regulation of development, glucose metabolism, and tissue regeneration. However, Lin28 overexpression is correlated with the onset of numerous cancers, whereas let-7, a tumor suppressor, silences several human oncogenes. Lin28 binds to precursor let-7 (pre-let-7) hairpins, triggering the 3′ oligouridylation activity of TUT4/7. The oligoU tail added to pre-let-7 serves as a decay signal, as it is rapidly degraded by the exonuclease Dis3L2. Genetic disruption of Dis3L2 is the primary cause of Perlman syndrome, a congenital disorder leading to fetal overgrowth and an increased susceptibility to Wilms’ tumor development. Subsequent studies have shown that Wilms’ tumors, a common pediatric kidney cancer, overexpress Lin28, underscoring the role of miRNA regulation in kidney tumorigenesis. In somatic cells, in the absence of Lin28, TUT4/7 promotes let-7 biogenesis by catalyzing single uridine addition to a subset of pre-let-7 miRNAs. We are studying the molecular basis and mechanism of Lin28-mediated recruitment of TUT4/7 to pre-let-7, and its effect on the uridylation activity of TUT4/7, switching it from a monouridylation activity to an oligouridylation, and the subsequent degradation of pre-let-7 by Dis3L2.

Small RNA and tRNA Surveillance by the CCA-Adding Enzyme

Transcription in eukaryotes produces a number of long non-coding RNAs (lncRNAs). Two of these, MALAT1 and Menβ, which are well-characterized long nuclear-retained noncoding transcripts that are involved in cancer progression and paraspeckle formation, generate a tRNA-like small RNA in addition to the mature lncRNA. The stability of these tRNA-like small RNAs as well as that of bona fide tRNAs...
is monitored by the CCA-adding enzyme. Just as for bona fide tRNAs, the trinucleotide CCA is posttranscriptionally added to the 3′ end of mascRNA (MALAT1-associated small cytoplasmic RNA), the tRNA-like transcript originating from the 3′ end of MALAT1. Unlike the acceptor stems of canonical tRNAs and mascRNA, the Menβ acceptor stem is destabilized through mismatches or wobble base pairs, somehow prompting the CCA-adding enzyme to repeat its catalytic cycle, thereby adding tandem CCA motifs. The CCACCA tail then serves as a degradation signal for the cellular RNA decay machinery. It appears now that many bona fide tRNAs that are destabilized through mutations or the lack of proper modifications are subjected to a similar CCACCA addition. Thus, the CCA-adding enzyme is not only critical for generating functional tRNAs but also likely plays a universal and central role in tRNA and tRNA-like small RNA surveillance and quality control.

We investigated how the CCA-adding enzyme distinguishes structurally stable from unstable RNAs so as to only mark unstable RNAs with CCACCA. We unraveled how a second CCA cycle can occur despite the enzyme possessing a unique mechanism that normally ensures a single cycle of CCA synthesis. We found that after the first CCA cycle, nucleotide binding to the active site induces the enzyme to apply torque on the RNA. A clockwise screw motion of the enzyme’s catalytic domain leads to RNA compression and overwinding. This causes unstable RNAs to extrude a distinctively positioned bulge from the acceptor stem while still bound to the enzyme. The bulge does not perturb the double-helical nature of the substrate, and all other structural determinants near the active site remain in place. The catalytic mechanism is thus preserved between the first and second cycles of CCA addition. In total, we found that tandem CCA addition is not the result of a modified enzymatic activity that is particular to unstable RNAs; rather, it is a consequence of the natural activity of the CCA-adding enzyme on a substrate with increased conformational flexibility. By exploiting the versatility of RNA structure, the CCA-adding enzyme is able to trigger the degradation of potentially detrimental small RNAs and tRNAs.

PUBLICATIONS


RNA SPLICING

A.R. Krainer  M. Akerman  H.-Y. Jeon  L. Manche  M. Rahman
O. Anczuków  V. Kergourlay  S. Meier  J. Scharner
I. Aznarez  Y. J. Kim  T. Nomakuchi  M. S. Wong
W.-J. Chen  K.-T. Lin  J. Novatt  M. S. Wong
Y. Hua  Y.-H. Liu  E. Panagot

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 signaling pathways. The fact that multiple protein isoforms can be expressed from individual genes shows 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.

Spliceosomal Protein and RNA Interaction Network

During spliceosome assembly, protein–protein interactions (PPIs) are sequentially formed and disrupted to accommodate the spatial requirements of pre-mRNA substrate recognition and catalysis. Splicing activators and repressors, such as SR proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs), modulate spliceosome assembly and regulate alternative splicing. However, it remains unclear how they differentially interact with the core spliceosome to perform their functions.

We investigated the protein connectivity of SR and hnRNP proteins to the core spliceosome using probabilistic network reconstruction based on the integration of interactome and gene-expression data. We validated our model by immunoprecipitation of the prototypical splicing factors SRSF1 and hnRNPA1, followed by mass spectrometry (with Dr. Darryl Pappin, CSHL). Network analysis revealed that a splicing factor’s properties as an activator or repressor can be predicted from its overall connectivity to the rest of the spliceosome. In addition, we discovered and experimentally validated PPIs between the oncoprotein SRSF1 and members of the SF3 subcomplex of U2 snRNP that are targeted by spliceostatin, pladienolide, sudemycin, and related antitumor drugs. Our findings suggest that activators tend to promote the formation of PPIs between spliceosomal subcomplexes, whereas repressors mostly operate through protein–RNA interactions.
We also collaborated with a former postdoc (Dr. Eric Allemand, Institut Pasteur, Paris) in a study of U2 snRNP interactions. In vivo–assembled U2 snRNP copurified with a subset of chromatin proteins, including histones and remodeling complexes such as SWI/SNF. Moreover, an unbiased RNA interference (RNAi) screen revealed that alternative splicing is influenced by many chromatin factors, not all of which are physically associated with the spliceosome. These chromatin factors had context-specific effects on splicing, resulting in inclusion of certain alternative cassette exons and skipping of others. A direct assessment of the impact of chromatin on splicing using an in vitro cotranscriptional splicing assay with pre-mRNAs transcribed from a nucleosomal template showed that chromatin can influence the splicing competence of nascent pre-mRNPs.

**Alternative Splicing and Cancer**

Alternative splicing plays 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.

Splicing factor SRSF1 is up-regulated in human breast tumors, and its overexpression promotes transformation of mammary cells. Using RNA-seq, we identified SRSF1-regulated alternative-splicing targets in organotypic three-dimensional human MCF-10A cell cultures that mimic a context relevant to breast cancer. We identified and validated hundreds of endogenous SRSF1-regulated alternative-splicing events. De novo discovery of the SRSF1 binding motif reconciled apparent discrepancies in previous motif analyses derived by different methods. Nuclear magnetic resonance and isothermal titration calorimetry with SRSF1 RRM1 + RRM2 and wild-type or mutant versions of an RNA oligonucleotide comprising the motif confirmed the validity of the RNA-seq-derived SRSF1 motif (in collaboration with Dr. Frédéric Allain, ETH, Zurich). Using a Bayesian model, we determined positional effects of SRSF1 binding on cassette exons: Binding close to the 5′ splice site generally promoted exon inclusion, whereas binding near the 3′ splice site promoted either exon skipping or inclusion. Finally, we identified SRSF1-regulated alternative-splicing events deregulated in human tumors; overexpressing one such isoform, exon-9-included CASC4, increased MCF-10A acinar size and proliferation, and decreased luminal apoptosis, partially recapitulating SRSF1’s oncogenic effects on acinar morphogenesis. Thus, we uncovered SRSF1 positive and negative regulatory mechanisms and oncogenic alternative-splicing events that represent potential targets for therapeutics development.

**Targeted Antisense Modulation of Alternative Splicing or NMD**

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, full-length SMN protein. *SMN2* decreases the severity of SMA in a copy number–dependent manner. We previously developed an antisense oligonucleotide (ASO) that efficiently increases the extent of exon 7 inclusion during splicing of *SMN2* transcripts for therapeutic use in SMA. This translational research was performed in collaboration with Ionis Pharmaceuticals. After extensive preclinical testing, and phase I and II open-label clinical trials, phase III randomized, double-blind, sham-procedure-controlled clinical trials with the ASO compound, nusinersen, are well under way (sponsored by Ionis and Biogen). The drug, which is administered to SMA infants and children by lumbar puncture, has been well tolerated at all doses tested, and dose- and time-dependent improvements in motor function have already been observed in the open-label studies.

Although it is clear that SMN deficiency causes SMA, the pathogenesis mechanisms remain elusive. SMN is ubiquitously expressed and is an essential protein, but its reduction appears to selectively affect motor neurons for unknown reasons. However, selectively
restoring SMN in motor neurons only partially rescues SMA in mouse models. We addressed the relative importance of SMN restoration in the central nervous system (CNS) versus peripheral tissues in mouse models, using a systemically administered therapeutic splice-switching ASO to broadly restore SMN and an intracerebroventricularly administered complementary decoy oligonucleotide to neutralize the effects of the therapeutic ASO within the CNS. We could show that this procedure effectively cancels out the effect in the CNS of ASO that crosses the immature blood–brain barrier in neonate mice. Remarkably, increasing SMN exclusively in peripheral tissues completely rescued necrosis in mild SMA mice and robustly extended survival in severe SMA mice with significant improvements in vulnerable tissues and motor function, as well as preservation of spinal motor neuron counts and neuromuscular-junction integrity. Our data show a critical role of peripheral pathology in the mortality of SMA mice and indicate that peripheral SMN restoration compensates for its deficiency in the CNS and preserves motor neurons. Thus, SMA is not a cell-autonomous defect of motor neurons in SMA mice.

SMN is expressed ubiquitously and functions in RNA processing pathways that include trafficking of mRNA and assembly of snRNP complexes. Importantly, SMA severity is correlated with decreased snRNP assembly activity. In particular, the minor spliceosomal snRNPs are affected, and some U12-dependent introns have been reported to be aberrantly spliced in patient cells and animal models. It is likely that aberrant splicing of genes expressed in motor neurons is involved in SMA pathogenesis, but as mentioned above, increasing evidence indicates that pathologies also exist in other tissues. We collaborated with Dr. Brage Andresen (University of Southern Denmark, Odense) to comprehensively analyze multiple tissues from an SMA mouse model by RNA-seq. This study showed elevated U12-dependent-intron retention in all tissues examined, and such minor-intron retention was also observed on siRNA knock down of SMN in HeLa cells. Furthermore, retention of minor introns was reversed by ASO treatment to restore SMN levels in SMA mice, as were many downstream transcriptional changes. Finally, we proposed that missplicing of several minor-intron-containing ion-channel genes might account for disrupted calcium homeostasis in SMA.

We also collaborated with Dr. John Staropoli (Biogen) to characterize gene-expression changes in an induced mouse model of SMA, with or without ASO therapy. This study involved a technique we previously published, TSUNAMI, which uses an ASO to promote exon 7 skipping in SMN2-transgenic mice and, thus, phenocopy SMA in a dose-dependent manner, followed by rescue with a different ASO to restore exon 7 inclusion. The observed gene-expression changes in the CNS of the induced model of SMA were substantially rescued on intracerebroventricular administration of the therapeutic ASO, with earlier administration promoting greater rescue. This study generated a robust reference set of preclinical pharmacodynamic gene-expression effects for comparison with other investigational therapies for SMA.

Another area in which we combined basic knowledge of RNA-processing mechanisms with antisense technology to develop a novel therapeutic approach is NMD. NMD is a cellular quality-control mechanism that is thought to exacerbate the phenotype of certain pathogenic nonsense mutations by preventing the expression of semifunctional proteins. NMD also limits the potential efficacy of therapies based on translational read-through compounds, because it causes a reduction in the mRNA template available for translation. NMD depends in part on exon-junction complexes (EJCs), which are assembled upstream of exon–exon junctions on completion of splicing in the nucleus. The pioneer round of translation in the cytoplasm displaces bound EJCs until the ribosome reaches the natural stop codon or a premature stop codon (PTC). In the latter case, the presence of one or more bound EJCs downstream from the PTC-bound ribosome constitutes a signal that initiates mRNA decay.

We developed a gene-specific method of NMD inhibition using ASOs to block EJC deposition downstream from a PTC. Using various target genes with nonsense mutations, we systematically screened sets of ASOs complementary to the region spanning the expected site of EJC deposition, looking for those that resulted in mRNA stabilization, allowing increased expression of truncated protein. We then combined this approach with an aminoglycoside read-through compound to effectively restore the expression of full-length protein. We verified the mechanism of action by showing that the effective ASOs blocked EJC binding in an in vitro splicing reaction, as well as by demonstrating...
the position dependence of the ASO effect with respect to the downstream exon–exon junction. Finally, we showed that two ASOs targeting consecutive EJCps could be combined to stabilize an mRNA with a nonsense mutation in the second-before-last exon.

**PUBLICATIONS**


**In Press**


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. Over the past year, our research has continued to focus on identifying and characterizing the role of long noncoding RNAs (lncRNAs) in cancer progression and/or differentiation. Following is an overview of some of our major accomplishments during the past year.

Identification of lncRNAs Involved in Breast Cancer Progression
G. Arun, S. Diermeier, K.-C. Chang, S. Russo, S. Hearn, N. El-amine [in collaboration with F. Rigo, R. MacLeod, C. Frank Bennett, Ionis Pharmaceuticals]

The mammalian genome encodes a huge repertoire of noncoding RNAs. MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is among the most abundant nuclear-enriched IncRNAs. MALAT1 is encoded at 11q13.1 in the human genome and its expression is up-regulated in many cancers including breast, lung, prostate, and hepatocellular carcinoma. MALAT1 is up-regulated at least three- to fourfold in human mammary tumors, as well as different human mammary tumor cell lines, compared with normal tissue/cell samples. Up-regulation of MALAT1 has been correlated with high metastatic progression and poor prognosis. In addition, MALAT1 gene mutations have been shown to be recurrent in luminal-type breast tumors (Ellis et al., Nature 486: 353 [2012]). However, the mechanism by which MALAT1 functions and whether it is a driver in breast cancer progression remain largely unclear. Our efforts are aimed at addressing the role of MALAT1/Malat1 in metastatic breast cancer.

RNA-FISH (fluorescence in situ hybridization) experiments on primary tumors and metastatic nodules from patients with luminal-type breast cancer revealed that MALAT1 IncRNA is up-regulated three to four times in metastatic nodules as compared with primary tumors. This is consistent with the possibility that MALAT1 plays an important role in the metastatic progression of luminal breast cancers. We have used the MMTV-PyMT mouse model of luminal B breast cancer to characterize the role of Malat1 in primary breast cancer and its subsequent metastasis. Malat1 IncRNA was knocked down via subcutaneous administration of antisense oligonucleotides (ASOs) at a dose of 50 mg/kg/3×/wk over a period of 7 wk, after which animals were killed and primary tumors and lungs were removed for molecular and histological analyses. Malat1 ASO treatment resulted in ~60% knockdown in the primary tumor concomitant with a significant reduction in tumor progression rate, as well as a change in the differentiation status of the primary tumor. Detailed histopathological analysis of ASO-treated tumors showed an increase in well-differentiated ductular tumors, whereas scrambled ASO-treated tumors progressed to solid undifferentiated carcinomas. Most interestingly, a marked decrease was observed in the incidence of lung metastases: ~70% fewer metastatic nodules in Malat1 ASO-treated animals than scrambled ASO-treated animals. Further, Malat1 ASO-treated ex vivo–generated mammary tumor organoids from MMTV-PyMT mice resulted in an inhibition of branching morphogenesis, which recapitulates aspects of the invasive process that initiates metastases in vivo. RNA-Seq (RNA-sequencing) analysis of the primary tumors and tumor-derived organoids treated with Malat1 ASO showed up-regulation of genes involved in differentiation and down-regulation of genes involved in migration and proliferation.
Over the past year, we have performed detailed bioinformatics analysis of the genes and pathways that are misregulated on Malat1 knockdown versus the control. KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis showed that cell adhesion and focal adhesion genes were significantly altered in the Malat1 ASO-treated tumors, as well as organoids. Additionally, extracellular matrix (ECM) interactions and cytokine signaling were also affected in the ASO-treated tumors. These data point to a defective protumorigenic ECM-intracellular signaling on Malat1 loss in tumors. Many integrins that are up-regulated during tumor progression were found to be down-regulated according to the pathway analysis. Further gene set enrichment analysis (GSEA) showed that the Malat1 knockdown dataset was similar to a number of datasets that were generated from alterations affecting tumor progression. For example, the majority of the up-regulated genes in Malat1 knockdown tumors were up-regulated in well-differentiated grade 1 tumors compared with grade 3 tumors. To identify the transcription factors potentially involved with Malat1, we performed both known and de novo motif analysis of the promoter region (–900 to +100 in relation to the transcription start site [TSS]) of the genes that showed altered expression on Malat1 knockdown. Interestingly, these genes showed significant enrichment in Sox5-, Tcfcp2l1-, and E2A-binding motifs in their promoter region. In addition, we took a sequence-independent approach by searching for overlaps between Malat1 putative targets and >359 chromatin immunoprecipitation (ChIP)-seq and ChIP-ChIP experiments annotated in the ChEA database. From a total of 140 transcription factors, Sox9 was identified as a top scorer ($p = 0.0002$). Sox9 is a high mobility group (HMG) protein that shares a similar DNA-binding element that was predicted for Sox5 binding in our motif analysis. Studies are currently under way to examine the potential interactions of Malat1 and Sox proteins in regulating tumor gene expression.

In addition to our efforts in regard to Malat1 IncRNA, we generated a comprehensive compendium of lncRNAs that show overexpression in mammary carcinomas compared with the normal mammary gland epithelium and, as such, represent potential therapeutic targets. Thus far, we performed an unbiased RNA-Seq screen on three physiologically relevant transgenic mouse models of luminal B (MMTV-PyMT) and HER2/neu-amplified (MMTV-Neu-NDL and MMTV-Cre;Flox-Neo-Neu-NT) subtypes of human breast cancer. Over the past year, we updated our computational analysis to the latest GENCODE M7 annotation set. Thereby, we increased our list of differentially regulated lncRNAs to a total of 290 lncRNAs that are up-regulated at least twofold compared with normal mammary glands. From these 290 up-regulated lncRNAs, we selected 30 previously uncharacterized transcripts for further evaluation. These transcripts were termed mammary tumor-associated RNA 1-30 (MaTAR1-30). Interestingly, 20 MaTARs show either exclusive expression or strong up-regulation in mammary tumors compared with any other adult organ. To further characterize
MaTARs in the context of global gene expression and to elucidate their potential as “driver genes” in mammary carcinogenesis, we performed weighted gene correlation network analysis (WGCNA). Remarkably, 17 of the 30 MaTARs are residing within the same network module. To identify key drivers of this module, we focused on potential “hub genes” with high intramodule connectivity (>1550) and very good correlation with the module eigengene (≥0.9). At least six MaTARs meet the applied criteria, indicating their importance within the module.

Based on the results of our computational approach, we performed knockdown experiments of 14 MaTARs using ASOs in primary MMTV-PyMT tumor cells. Depending on the target, knockdown efficiencies ranged from 38% to 89% after 24 h. To investigate the functional impact of MaTAR down-regulation on tumor cells, we combined the ASO treatment with cell viability or invasion assays. Interestingly, MTT assays revealed that the knockdown of five candidates led to a 20%–50% decrease of cell viability. In addition, we performed invasion assays of tumor cells in 96-well Boyden chamber plates on ASO-mediated knockdown. We detected a significant reduction of tumor cell invasion on knockdown for six MaTARs (20%–49%).

To further elucidate the functional role of MaTARs in a more physiological context, we performed ASO-mediated knockdown experiments in mammary tumor organoids. We observed knockdown efficiencies in organoids ranging from 30% to 68% after 6 d of ASO treatment. Culturing the organoids for several days allowed us to observe any phenotypic changes that might be caused by the knockdown. We detected a distinct decrease (20%–50% less compared with untreated organoids) in duct formation for 14 MaTARs. To further support the role of MaTARs as key genes in tumor progression, we performed independent ASO-mediated knockdown of three MaTARs in control organoids derived from WT nulliparous mammary glands. Importantly, we did not see a pronounced loss of branching in the normal mammary gland organoids, strongly indicating the cancer-specific role of these three lncRNAs.

Based on our in vitro results, we chose the five most promising MaTARs and evaluated their therapeutic potential in vivo by subcutaneous injections of specific ASOs (50 mg/kg/3×/wk) into four to five female MMTV-PyMT mice. Tumor growth rates were monitored for up to 9 wk, and effects on tumor volume and morphology were investigated with respect to nonsilenced control mice injected with a scrambled ASO. Animals were killed as soon as one of the tumors reached 2 cm in diameter. Interestingly, independent knockdown of three MaTARs significantly reduced tumor growth (up to 40%). Primary tumors were isolated and analyzed regarding their morphological and histological properties using hematoxylin and eosin (H&E) staining of tissue sections. We observed moderate to severe tumor necrosis on treatment with ASOs independently targeting two MaTARs and increased differentiation in tumors treated with ASOs targeting a third MaTAR. In the case of the MMTV-PyMT mouse model, the lungs of ASO-treated mice were examined regarding the number and size of metastatic nodules compared with control animals. Notably, treatment with ASOs targeting one MaTAR resulted in an 80% decrease of metastatic nodules. Ongoing studies are pursuing the molecular function of these MaTARs and their potential as therapeutic targets in metastatic breast cancer.

Characterization of lncRNAs Associated with Pluripotency

A. Yu, R. Hazra [in collaboration with F. Rigo, Ionis Pharmaceuticals]

We performed high-depth poly(A)+ RNA-Seq across multiple clonal populations of mouse embryonic stem cells (ESCs) and neural progenitor cells (NPCs) to comprehensively identify differentially expressed intergenic lncRNAs (Bergmann et al. 2015). Out of the 3506 genes annotated in GENCODE M3 as “lincRNA” or “processed transcript” (Ensembl v.76; referred to as “lncRNA” for the remainder of this study), we detected 958 lncRNAs expressed at ≥0.1 FPKM in the union of our ESC or NPC datasets. This set of lncRNAs contained more than 800 that are largely uncharacterized on the molecular level. Of the 800 lncRNAs identified, 508 (64%) displayed a significant change in expression level on differentiation of ESCs to NPCs (DESeq2, FDR < 0.01), with 439 lncRNAs being exclusively detected in either cell type. We also used ab initio transcript assembly by means of Cufflinks to assess the completeness of the GENCODE annotation. From our analyses, we
prioritized 50 lncRNA candidates that showed expression in ESCs, thus potentially implicating them in the coregulation of self-renewal or pluripotency-related networks. Because we are interested in identifying lncRNAs whose expression is strongly associated with the ESC state, we also analyzed raw poly(A)⁺ RNA-Seq data from 22 mouse tissues (including fetal liver and neural tissues of embryonic origin) recently released by the ENCODE project. Gene-level analysis of this matrix showed that the majority of the 50 lncRNAs most specifically expressed in ESCs displayed exclusive expression in this cell type, rendering them viable candidates as pluripotency biomarkers. Ongoing studies are analyzing the functional associations of several of these ESC- and NPC-specific lncRNAs.

lnc05 is a particularly interesting lncRNA as in addition to being up-regulated in mouse ESCs it is also enriched in embryonic, but not adult, liver. As there are overlaps between pluripotency and cancer with respect to self-renewal properties, we decided to focus on the mouse hepatocellular carcinoma (HCC) cell line HepA1-6. Interestingly, we found that lnc05 is up-regulated 10-fold in HepA1-6 cells compared with normal mouse hepatocytes. Knocking out lnc05 in HepA1-6 cells (Δlnc05) increases the doubling time of these cells by 23% and modulates the fraction of cells in S and G₂ phases of the cell cycle. Knockdown of lnc05 expression using ASOs also decreases proliferation, as assessed by expression of Ki67, a proliferation marker, and colony formation assays. We are currently exploring the potential role of lnc05 in HCC using mouse models and ASO treatment to determine the impact of lnc05 loss or overexpression on HCC. In addition, we are assessing the potential role of lnc05 in human HCC by comparing liver samples from individuals with normal or minimal changes in the liver (which are deemed “healthy” individuals), samples from individuals with cirrhosis, and samples from paired HCC tumor and adjacent nontumoral cirrhotic liver tissues. This analysis will provide insight into the role of lnc05 in HCC and assess its potential as a therapeutic target to treat this devastating disease.

PUBLICATIONS


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

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

The Amino-Terminal Domain in E1 Controls the Formation of an E1 Double Trimer (DT) and the Transition to the Double Hexameric (DH) E1 Helicase

The papillomavirus E1 protein is a representative of a subgroup of hexameric helicases. However, E1 is not a dedicated helicase but serves several additional functions in the viral life cycle. E1 takes part in initiation of viral DNA replication in several different ways: The E1 DNA binding domain provides sequence-specific binding to the viral origin of replication (ori). A particular form of E1, a DT, can then generate local melting of the viral ori. Once the DNA is melted locally, an E1 DH is formed on the melted DNA and unwinds the DNA in front of the replication fork.

Obviously, the utilization of a single polypeptide to provide multiple functions presents many challenges.
In addition to accommodating multiple different activities in one polypeptide, mechanisms have to exist that allow switching between the different activities, as well as mechanisms that prevent the different activities from interfering with each other. For example, in the DT to DH transition, E1 switches from site-specific DNA binding and complex formation dictated by E1 binding sites in the ori to formation of hexamers dictated by the oligomerization domain and binding to ssDNA. How this feat is accomplished is very poorly understood.

We have previously demonstrated that the E1 protein contains at least two DNA binding activities. One of these is the E1 DBD, which binds to specific sites in the origin of DNA replication and directs binding of E1 as a head-to-head dimer. The second DNA binding activity is composed of a β-hairpin structure in the helicase domain, which directs non-sequence-specific DNA binding. These elements are required to form the DT and the DH complexes. In addition to the DT and DH complexes that form on the origin of DNA replication, E1 can form two sequence-independent complexes on double-stranded DNA. These complexes, a trimer in the presence of ADP and a hexamer that depends on ATP hydrolysis, provide information about the intrinsic ability of E1 to oligomerize and are thought to relate to the formation of the DT and DH, respectively.

We wanted to determine whether the formation of the trimer and hexamer also relies on the same two DNA binding elements that are important for formation of the DT and DH. We therefore generated mutations in the DBD and in the β-hairpin in the helicase domain in residues involved in DNA contacts and tested these for trimer and hexamer formation. Strikingly, although the β-hairpin was required for trimer formation, the DBD was not. However, the DBD was critically important for hexamer formation. Because the β-hairpin by itself does not account for trimer formation, we examined E1 for additional DNA binding activities. We were able to identify a short, highly conserved peptide in the amino-terminal domain that bound DNA nonspecifically and was required for trimer formation but did not play a role for hexamer formation. These results demonstrate that the trimer and hexamer represent two fundamentally different ways of binding to DNA.

Interestingly, the amino-terminal DNA binding activity, which clearly is required for DT formation, when exposed also inhibits DH formation. This inhibition is released when ATP is hydrolyzed. This new understanding of E1 complex formation on DNA now will make it possible to recapitulate the changes in E1 DNA binding that accompany the transition from DT to DH.

**PUBLICATION**

For the past three decades we have been focusing on identifying the cellular proteins that are required to duplicate the DNA in the chromosomes of eukaryotic cells. Essentially, all of the key proteins have been identified and some stages of the process of DNA replication have been reconstituted in vitro with purified proteins, particularly the establishment of pre-replicative complexes (pre-RCs) at origins of DNA replication and the initiation of DNA replication. In addition to understanding the control of genome replication in the yeast *Saccharomyces cerevisiae*, a main focus has been to identify replication proteins from yeast, determine their function, and then identify and characterize their homologs in human cells so that comparative analyses can be performed. Increasingly, we have focused on structural studies of the yeast initiation proteins and the various initiation assembly intermediates using cryo-electron microscopy in collaboration with Dr. Huilin Li at Brookhaven National Laboratory and former postdoctoral fellow Dr. Christian Speck, now at Imperial College in London. Biochemical studies of the more complicated and functionally diverse origin-recognition complex (ORC) from human cells is uncovering new activities for ORC, including roles in processes of chromosome replication and segregation, which include the initiation of DNA replication, assembly and maintenance of heterochromatin, centriole and centrosome duplication, kinetochore function, and cytokinesis. We have recently discovered that human ORC is also involved in controlling the transcription of the gene encoding cyclin E-CDK2, a key cell division cycle regulatory protein that is involved in the process of committing cells to replicate and segregate DNA before cell division.

**Whole-Genome Studies of Replication in Checkpoint Mutants**

A number of years ago we showed that the unstructured domain of the amino terminus of the Mcm4 protein (called the NSD) is targeted by multiple protein kinases, including the Cdc7-Dbf4 protein kinase (called DDK) and the S-phase-specific cyclin-dependent protein kinase (called S-CDK). Mcm4 is an essential subunit of the replication helicase hexameric Mcm2-7 complex, which is loaded onto chromosomes during G1 phase and then activated during S phase to participate as a component of the replicative helicase. In addition to CDK and DDK kinases that are required for activating the initiation of DNA replication, two other protein kinases, Mec1 and Rad53, block the initiation of DNA replication when cells experience profound DNA damage or DNA replication stress. Activation of Mec1 and Rad53 kinases prevents further cell DNA replication during S phase until the damage is fixed by blocking those origins of DNA replication that have not yet initiated DNA replication. These so-called checkpoint kinases also send signals to prevent mitosis until DNA replication has been completed. The Mec1 kinase is known to phosphorylate the Mcm4-NSD, but the physiological function of this phosphorylation is not known.

When a segment of the Mcm4 NSD (amino acids 74–174 of the 933 amino acid Mcm4 protein) was deleted, cells no longer required DDK for the initiation of DNA replication, suggesting that Mcm4 is an essential regulatory target of DDK. Treatment of cells with hydroxyurea (HU) prevents the efficient synthesis of deoxyribonucleoside triphosphates (dNTPs), a stress that limits DNA replication and induces the checkpoint kinases Rad53 and Mec1. Normally, blocking dNTP synthesis arrests DNA replication and all origins of DNA replication that have not become active when the stress was applied fail to initiate DNA replication until the stress is relieved. We found, however, that under limited dNTP synthesis conditions in cells treated with HU, removing the Mcm4-NSD initiation inhibitory region partially activated those origins of DNA replication that would normally be blocked by the stress conditions. In parallel, others had shown...
that under the same replication stress conditions, the checkpoint kinase Rad53 phosphorylated both the Dbf4 protein, a regulatory subunit of DDK, and an essential initiation protein called Sld3, and this phosphorylation inhibited their activities. When, however, mutant versions of Dbf4 and Sld3 that could no longer be phosphorylated by Rad53 were introduced into cells, cells partially activated those origins that would normally be suppressed by HU. Because the Dbf4 and Sld3 mutations and the Mcm4-NSD mutations by themselves only partially activated replication origins under replication stress, we tested whether combining all three mutations into a single cell would activate all origins in the presence of HU as efficiently as removing Rad53 kinase or Mec1 kinase. The result showed that removing the combined regulatory activities of the Dbf4, Sld3, and Mcm4 proteins was almost identical to removing the Rad53 or Mec1 checkpoint kinases. These results suggest that when a cell experiences DNA damage or DNA replication stress during S phase of the cell division cycle, DNA replication is blocked by inhibiting the activities of Dbf4 and Sld3 and preventing the inactivation of the Mcm4-NSD regulatory domain. The role of the Mcm4 NSD in regulating late origin firing and fork progression in HU was largely recapitulated in cells replicating in methyl methane sulfonate (MMS), an alkylating agent and a carcinogen that damages DNA. Although there are differences in the response by DNA replication to treatment with HU versus MMS, the results from our recent studies, those from previous years and those of others suggest a model for control of the initiation of DNA replication, both positive and negative (Fig. 1). Positive activation of DNA replication by the CDK and DDK kinases is required to trigger the initiation of DNA replication from preformed pre-RCs when cells are ready to replicate the genome. On the other hand, if cells encounter conditions such as DNA damage or replication stress, genome replication is halted by the Mec1 and Rad53 kinases blocking the activities of Dbf4 (a component of DDK), Sld3 (a key initiation protein), and allowing the intrinsic Mcm4 initiation inhibitor to remain active. In this manner, cells can repair the DNA damage or recover from the replication stress without attempting to copy the DNA under conditions that would generate irreparable genome damage.

**PUBLICATIONS**


In Press


TRANSCRIPTIONAL REGULATION OF MAMMALIAN CELL GROWTH

C.R. Vakoc  A. Bhagwat  J. Milazzo  T. Somerville
A. Hohmann  J. Roe  Y. Tarumoto
Y. Huang  C. Shen  Y. Xu
B. Lu  J. Shi

Broad alterations in the rate of gene transcription are necessary, and often sufficient, for cells to undergo malignant transformation. As a consequence, cancer cells are vulnerable to perturbations of individual transcription regulators, including DNA-binding transcription factors and general cofactors. Our laboratory has taken a functional-genomics approach to identify essential transcriptional regulators in cancer cells in an effort to expose opportunities for therapeutic intervention. Our initial efforts used shRNA screening to identify the BET bromodomain protein BRD4 as a targetable non-oncogene dependency in acute myeloid leukemia. More recently, we have pursued the use of CRISPR-Cas9 genome editing as a tool for annotating essential transcriptional regulators in cancer. In the past year, we have advanced our fundamental understanding of BRD4 as a therapeutic target in leukemia, focusing on the upstream and downstream factors that support BRD4-dependent transcriptional activation. In addition, we are making extensive use of CRISPR-Cas9 to identify and validate therapeutic targets in cancer, exploiting the heterogeneity of Cas9-induced indel mutations to infer structure–function relationships of individual protein domains.

Characterization of Brd4 Function in Acute Myeloid Leukemia
A. Bhagwat, J. Roe, C. Shen [in collaboration with L. Joshua-Tor and D. Pappin, CSHL]

Original studies from our laboratory in 2011 identified the BET 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 and last year the first complete responses were reported in leukemia patients receiving these agents. 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 initial focus has been to define the key cis elements utilized by Brd4 to regulate its important target genes in leukemia cells. Using chromatin IP followed by next-generation sequencing, we have 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 super-enhancers, appear to be the functionally relevant mode of regulation by Brd4 for a subset of its target genes. For example, Myc is among the most Brd4-dependent genes for its expression, and Myc is located near one of the most profoundly Brd4-occupied enhancers in the leukemia genome. Interestingly, the Myc enhancers closely correspond to a region found previously as a site of recurrent focal amplification in leukemia patients, which has clarified the mechanism of transcriptional regulation performed by Brd4 to support leukemia maintenance.

An additional area of investigation has been to define the recruitment mechanism that directs Brd4 to specific cis elements in the leukemia genome. Through an epigenomic analysis of Brd4-occupied sites, we have uncovered a suite of hematopoietic transcription factors (Pu.1, Flt1, Erg, Myb, C/EBPα, C/EBPβ) that determine the locations of Brd4 occupancy. Remarkably, introducing these transcription factors into fibroblast cells can reprogram Brd4 occupancy into its leukemia-specific configuration.
This effect occurs through an ability of the transcription factors to recruit the lysine acetyltransferase proteins p300/CBP to their occupied sites, which in turn acetylates various protein substrates at enhancers and promoters to promote bromodomain-dependent Brd4 recruitment. One of the key substrates of p300/CBP is likely to be histone H3 and H4; however, we have also derived evidence that Erg acetylation might also facilitate Brd4 recruitment. Collectively, this work reveals a chromatin-based signaling cascade composed of hematopoietic transcription factors, p300/CBP, and Brd4, which supports leukemia maintenance and is suppressed by BET bromodomain inhibition.

Another area of mechanistic investigation has been to identify candidate effectors of Brd4 in facilitating transcriptional activation. Through immunoprecipitation coupled with mass-spectrometry analysis, we have identified several Brd4-associated proteins. By filtering of candidates through genetic criteria of having critical roles in leukemia maintenance, we have initiated studies of Nsd3, which is a SET domain-containing histone H3K36 methyltransferase that has not been well characterized previously. In ongoing studies, we have found that Nsd3 functions as a bona fide effector for Brd4 in supporting leukemia maintenance. We find that the genome-wide localization of Nsd3 matches that of Brd4, and that chemical inhibition of BET bromodomains leads to displacement of Nsd3 at promoter and enhancer regions. Unexpectedly, we have discovered that Nsd3 performs its essential function in leukemia cells independently of its SET domain, and we are currently investigating how Nsd3 promotes transcriptional activation independently of its catalytic domains.

We have also found that Brd4 utilizes the mult-subunit Mediator complex as an effector at a subset of its occupied sites, which includes the Myc enhancers. Remarkably, a short treatment with BET inhibitors causes a large-scale displacement of several Mediator subunits from the Myc enhancers and from various other regulatory sites in the genome. In addition, we find the genetic perturbation of several Mediator subunits leads to leukemia cell differentiation, which mimics the effects of targeting Brd4. Collectively, these studies suggest that Brd4 facilitates Mediator recruitment to promote transcriptional activation of certain target genes. This represents progress in defining downstream components of Brd4 function that are functionally suppressed by BET bromodomain inhibition.

**Function and Inhibition of Brd9-SWI/SNF Complexes in Acute Myeloid Leukemia**

A. Hohmann

Prior research from our laboratory has implicated the SWI/SNF complex as a unique vulnerability in acute myeloid leukemia. In the absence of any available drugs that target SWI/SNF, we have pursued the identification of therapeutic approaches that would be capable of modulating SWI/SNF function in leukemia cells. Based on proteomic and genetic evidence, we have identified one SWI/SNF subunit, called Brd9 (bromodomain containing protein 9) as an attractive target for chemical inhibition. More specifically, our genetic evidence has implicated the bromodomain pocket of Brd9 as supporting leukemia maintenance and, based on this finding, chemistry collaborators have initiated the development of selective small-molecule inhibitors of Brd9. Notably, these inhibitors limit leukemia cell proliferation and mimic many of the cellular effects of RNAi-based Brd9 inhibition. Furthermore, we have engineered a mutant Brd9 cDNA that no longer binds to the small molecule but retains functionality. Using this novel reagent, we have shown that Brd9 is the sole cellular target of this novel inhibitor that is responsible for antileukemia effects. Having established genetic and chemical evidence to support Brd9 as a drug target in leukemia, we now seek to advance Brd9 inhibition as a therapeutic strategy in leukemia. To this end, we are pursuing experiments that define the molecular function of Brd9 in leukemia cells, thus revealing how Brd9 contributes to the overall functionality of the SWI/SNF complex and to the maintenance of a leukemia-supporting gene regulatory network. In addition, we are investigating the genetic basis for primary sensitivity and resistance to Brd9 inhibition using existing human acute myeloid leukemia cell lines. These experiments will likely be complemented by CRISPR-based genetic screens that seek to identify genes that, when mutated, confer resistance to Brd9 inhibition, thus providing critical insight into adaptive resistance. Finally, we will perform preclinical trials of Brd9 inhibitors in genetically engineered mice and in human xenograft
models of disseminated acute myeloid leukemia. This research holds great promise in revealing basic mechanisms for an unstudied chromatin reader protein as well as a strong translational potential for exploring Brd9 inhibition as an epigenetic therapy in acute myeloid leukemia.

Trim33 Allows the Survival of B Lymphoblastic Leukemia Cells by Deactivating a Single Enhancer Element
E. Wang [in collaboration with J. Kinney, CSHL]

We have recently extended our epigenetic screening platform into the high-risk 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 genomewide 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.

A remarkable attribute of Trim33 in B-ALL is that it preferentially occupies a small number of cis elements across the genome. Two discrete regions are heavily occupied by Trim33, which includes a distal upstream enhancer located 117 kb upstream of the Bim promoter. We find that Trim33 is recruited by a dense cluster of PU.1, a hematopoietic transcription factor, which in turn deactivates the underlying enhancer. A stunning observation in this study is that CRISPR-based deletion of the Bim 117-kb element renders Trim33 dispensable for B-ALL survival. This indicates that Trim33 performs an essential function through a single genomic binding site. To our knowledge, this indicates a level of regulatory precision not observed previously for a mammalian regulator of transcription. Finally, our findings also nominate TRIM33 inhibition as a therapeutic strategy in B lymphoid malignancies.

TFIID as a Leukemia-Specific Coactivator Dependency
Y. Xu

A major theme in our research investigating chromatin regulators as cancer dependencies is that major regulatory hubs in transcriptional control can be perturbed in a selective manner to eradicate cancer cells, which reflects the essential role of such machineries to implement oncogenic transcriptional programs. Based on this rationale, we have begun to probe the requirement of TFIID in cancer maintenance. TFIID is a multisubunit complex composed of TATA-binding protein (TBP) as well as various TBP-associated factors (TAFs) that function in promoter sequence recognition and as a coactivator for DNA-binding transcription factors. We have hypothesized that specific cancers might be hypersensitive to perturbation of certain TAF subunits of TFIID to elicit selective transcriptional effects on cancer-relevant genes. Custom shRNA libraries have been constructed targeting each TFIID subunit and introduced broadly into an assortment of different cancer cell lines. Out of this comparative screening has come the identification of a submodule of TFIID that uniquely supports the biology of leukemia. In future experiments, we will define genetically and biochemically how this submodule drives leukemia pathogenesis.

Transcriptional Pathway Discovery Using Domain-Focused CRISPR Screening in Leukemia, Sarcoma, and Epithelial Cancers
Y. Huang, B. Lu, J. Shi, J. Milazzo, T. Somerville, Y. Tarumoto

CRISPR-Cas9 technology holds great promise for genetic screening and for the discovery of therapeutic targets. To achieve optimal performance in negative selection screens, it is critical for CRISPR to generate homozygous loss-of-function mutations in a highly efficient manner. We have developed a CRISPR strategy that exploits this principle and simultaneously reveals protein domains that support cancer maintenance. By targeting CRISPR-mediated mutagenesis to exons encoding functional protein domains, negative selection phenotypes are achieved that are an order of magnitude stronger than those observed through mutagenesis of 5′ exons. We have also developed a deep sequencing-based method for target validation that effectively excludes off-target effects. We have constructed a diverse array
of domain-focused CRISPR libraries to enable discovery of transcriptional regulator dependencies in diverse forms of cancer, including hematopoietic, sarcoma, and epithelial malignancies. A major ongoing focus concerns the identification of novel transcriptional pathways that sustain the pathogenesis of cancer.

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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 hardwired, 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 rewiring 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.

The Je H. Lee lab studies how cells interact with their microenvironment to regulate gene expression during development. Single-cell heterogeneity in gene expression can result from spatial differences in cell–cell and cell–extracellular matrix interactions. Such differences contribute to stochastic evolution of tumor cells as well as morphogenesis during normal development. However, the spatial control of gene expression in complex tissues, embryos, or tumors remains poorly understood, because most genome-wide studies sample bulk tissues or dissociated single cells.

The Lee lab has recently developed a method to sequence RNA molecules directly within single cells and tissues using subcellular resolution imaging and demonstrated subtle differences in cell–cell/ECM signaling and gene expression genome-wide in situ. By clustering transcripts into functionally or morphologically discrete regions, they found many unique spatial markers and signaling pathways. The lab focuses on the role of noncoding RNA in chromatin remodeling and tumor progression using mouse and organoid models of human cancer. They use in situ sequencing, cell lineage tracing, and single-cell profiling to understand how noncoding RNA affects tumor cell evolution in their native context. The lab’s long-term goal is to develop better tumor classification tools and anticancer therapeutics using their understanding of the tumor microenvironment.

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 that 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.

Although aneuploidy—abnormal chromosome number—is a ubiquitous feature of human tumors, it occurs rarely in somatic cells. Thus, differences between aneuploid and euploid cells may represent crucial therapeutic vulnerabilities in cancer. Despite the prevalence of aneuploidy in cancer, its functional consequences for cell physiology remain poorly understood. The work of Jason Sheltzer and colleagues has demonstrated the existence of several surprising phenotypes that are shared among cells with different chromosomal imbalances. They have demonstrated that aneuploidy can function as a novel source of genomic instability, as aneuploid cells tend to display elevated levels of mutation, mitotic recombination, and chromosome loss. They have also identified a transcriptional signature of aneuploidy that is associated with cellular stress and slow proliferation and is found in aneuploid primary and cancer cells across a host of organisms. More recently, Sheltzer has investigated the link between aneuploidy and cellular transformation, demonstrating that aneuploidy can paradoxically function as a barrier to tumor growth. The lab is applying CRISPR-Cas9-mediated genome engineering to develop novel mouse models to explore the impact of gene dosage alterations on tumor development in vivo.

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 in 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.
We have two major focuses for our laboratory. The first project uses the model organism *Caenorhabditis elegans* to understand how normal gene regulation contributes to proper development, and how misregulation of expression leads to disease. Using forward genetic screens in our lab, we have made strides in identifying several novel genes and mechanisms that coordinate the timing of gene expression with the elaboration of normal temporal cell fate patterning. The first of these genes identified in this manner was *lin-42*, the *C. elegans* ortholog of the Period protein in humans and mice that negatively regulates oscillatory gene expression. In the last year, we have focused on identifying additional transcriptional components that generate the oscillatory expression patterns of critical regulatory genes that control temporal cell fate specification. The second half of the lab is focused on developing *C. elegans* as a model to understand how mutations of candidate genes implicated in autism spectrum disorder (ASD) and schizophrenia (SCZ) alter neuronal development. This project has identified the complex process of dendritic arborization as a common neuronal feature that is likely disrupted when the conserved genes implicated in ASD and SCZ are mutated.

Oscillatory Gene Expression and Developmental Patterning

K. Doerfel, C.M. Hammell

In normal development, the identity, behavior, and biological function of individual cells are coordinated with their neighbors to form distinct tissues. The defined structure and specification of tissues endows them with unique functional and physiological properties that exceed the capabilities of individual cells. Setting up these architectures is an incredibly complex process and requires aspects of spatial and temporal gene regulation to be coordinated to control facets of cell proliferation and cell fate determination. Although the last few decades have illuminated a variety of conserved regulatory mechanisms that mediate spatial gene regulation, our understanding of how temporal patterning is set up lags behind. From what we do know, the genes and mechanisms that control developmental timing are very well conserved, and disruptions in this regulation result in a variety of human diseases, including cancer.

In the past few years, we have used forward genetics and the *C. elegans* model to identify a number of genes that are essential for temporal gene expression. Mutations in these genes cause animals to alter their normally invariant cell lineage in one of two ways. Both of these phenotypes are referred to as heterochronic phenotypes as they change the relative timing or order of development. Specifically, some heterochronic mutations cause specific developmental events to be inappropriately reiterated, whereas other mutations cause individual developmental events to be skipped. These genes can further be classified according to whether their activity defines a stage-specific pattern of development or the gene normally functions to control transitions from one stage-specific pattern of development to the next. We are primarily interested in the latter class of mutants.

We found that many of the genes that we identified in our screens function at the transcriptional level and directly control the timing and the rates at which many regulatory RNAs (microRNAs) down-regulate suites of developmental genes. These observations led us to one of the most surprising findings from our lab, which is that a large fraction of the transcriptome, including the transcription of microRNAs, is expressed in a highly dynamic manner that is coupled to aspects of animal growth. Using the genetics of temporal cell fate specification and a variety of other molecular approaches, we are now characterizing the gene regulatory networks that generate these oscillatory transcriptional patterns.

Mutations in *blmp-1*, encoding a conserved ZnF transcription factor, result in mild heterochronic defects in which the expression of later developmental milestones is delayed. Using a variety of transcriptional...
green fluorescent protein (GFP) reporters, we showed that many of the microRNAs that are critical for temporal cell fate patterning are dependent on blmp-1 activity for their oscillatory transcriptional patterns. We generated stage-specific chromatin immunoprecipitation (ChIP)-seq data to determine where BLMP-1 is bound and presumably regulates the expression of downstream target genes. Consistent with the hypothesis that BLMP-1 normally functions to control the transcription of key microRNA genes, BLMP-1 was bound to the promoters of most microRNAs, which when mutant result in heterochronic phenotypes. In addition to binding the regulatory elements that are essential for normal microRNA expression, BLMP-1 was also bound to the putative regulatory regions of approximately 2500 coding genes.

To determine if BLMP-1 functions to regulate the expression of the approximately 3500 coding gene targets, we performed messenger RNA (mRNA)-seq experiments from samples collected throughout the development of wild-type and blmp-1 mutant animals. By performing independent component analysis of these expression patterns, these experiments allowed us to make several novel observations about transcriptional processes throughout *C. elegans* development. First, ~10%–12% of the transcriptome is expressed in an oscillatory fashion, which is tied to the molting cycles of larval growth. Second, genes that are expressed in a cyclical fashion can be divided into at least three functional groups that can be discriminated according to the phase of their expression in each cycle. And, finally, the BLMP-1 transcription factor is essential for...
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the oscillatory expression of one of these components (i.e., cyclic expression of these genes is lost in \textit{blmp-1} mutants) and the other two components are generated independently of BLMP-1 activity (Fig. 1).

**In Vivo Screening of Genes Implicated in Human Neuropsychiatric Disorders to Identify Genes Required for the Establishment or Maintenance of Neuronal Architecture**

C. Aquirre-Chen

High-throughput sequencing and genome-wide association studies of DNA derived from patients with various neuropsychiatric disorders has led to the identification of hundreds of separate mutations that are potentially causal. This rapid advance in the identification of inherited and de novo mutations for complex diseases like autism and SCZ has created a huge bottleneck for understanding the roles of these candidate genes in normal and disease processes. This is, in part, because of the sheer number of genes that have already been identified, the complexity of the nervous system itself, and the expense of initiating model organism-based strategy for understanding the disease process. What is needed to solve this problem is a rapid and cost-effective strategy that efficiently ascribes putative functions to individual candidate genes and also allows these lists to be organized into systems-level or molecular frameworks.

We recently developed an experimental platform in which conserved neuropsychiatric risk genes (NRGs) can rapidly and efficiently be tested in vivo for roles in establishing normal neuronal architecture. Our results indicate that many \textit{C. elegans} orthologs of ASD risk genes are essential for the establishment of normal dendritic arbors. Depletion of individual ASD

![Image](image.png)

**Figure 2.** The dendritic arbors of PVD neurons are highly structured and mutations in the \textit{C. elegans} orthologs of two “high value” ASD genes, \textit{ARID1B} and \textit{ANK2}, disrupt their development. (A–C) The dendritic arbors of adult animals are composed of repetitive units, known as a PVD “menorah” (B and C), that extend the entire length of the animal. These structures are easily visualized in living animals through the expression of a PVD-specific GFP reporter, \textit{wdls52}. (D–G) Mutations in \textit{let-526} and \textit{unc-44} (the \textit{C. elegans} orthologs of \textit{ARID1B} and \textit{ANK2}, respectively) result in distinct arborization defects (caret and arrows show a general disorder of dendritic arbors, whereas lower caret indicate severe dendritic hypobranching) and are highly quantifiable.
orthologs, via RNA interference (RNAi), results in distinct dendritic defects, which allowed us to both identify and characterize the regulatory or structural requirements that normally mediate arborization, as well as determine the genetic interactions between individual ASD risk genes. We are currently trying to expand these findings by designing a targeted protein degradation system to specifically modulate the expression of orthologous ASD genes with extreme temporal and spatial specificity. This will allow us to determine three aspects of candidate ASD function that were not addressed in our original RNAi-based strategy: (1) the cell types in which orthologous ASD genes function to control dendritic growth, (2) the exact temporal windows during dendritic development in which ASD orthologs are required, and (3) whether distinct candidate ASD genes are essential for the maintenance of dendritic arbors once normal architecture is established. Specifically, we are using CRISPR-Cas9 genomic editing to adapt an auxin-inducible degradation (AID) system in which the candidate ASD proteins can be specifically inactivated in a ligand- and tissue-dependent manner. Although this system can be used to characterize any of the genes we have previously identified via RNAi, we will benchmark this approach by dissecting the function of two “high-confidence” genes that are directly implicated in ASD via the Simons Simplex Collection.
Our lab is developing a cutting-edge method that can identify the location of gene transcripts genome-wide in vivo. In biology, both location and context matter. The spatial context of active gene expression in response to a limited number of signaling pathways governs critical morphological features in development, which becomes highly disorganized during cancer progression. How do cells at varying locations interpret these signals into highly diverse transcriptional programs, tissue patterns, and behaviors in a context-dependent manner? To use an analogy, a poetry verse can have different meanings to different people. To predict how readers will respond, it is essential to understand its cultural context and the audience’s background. The same is true for cells; we must understand a cell’s genetic, epigenetic, and environmental context in order to decipher the phenotypic consequence of cell signals from their neighbors.

Until now, high-resolution techniques to evaluate the ecological context of cells did not exist. The best methods available had relied on bulk measurements where masses of tissue are ground to a pulp or on random sampling of a few single cells without their location information. If Charles Darwin had arrived at the Galápagos Islands and blended different specimens together or randomly sampled them without noting the location of their habitat, the fundamental insight of how inheritable phenotypes coevolve on individual islands would not have been possible. To discover novel insights into how cells evolve and respond to biological signals, we believe that a different approach is necessary.

The Genetic Basis of Glioma Histopathology and Clonal Evolution

D. Ghosh [in collaboration with T. Chan, Memorial Sloan Kettering Cancer Center; M. Hammell, CSHL]

We are developing an innovative technique (fluorescent in situ sequencing [FISSEQ]) to study the gene expression in single cells within their original tissue architecture. In this technique, a tissue is preserved and loaded onto a microscope slide. The transcripts embedded within the tissue are transformed into readable DNA sequences at the exact site of operation. The DNA sequences can then be read using standard sequencing techniques and reconstructed into a 3D image of the original tissue. The result is an image that encodes information about which genes were active in different tissue regions with distinctive features. We can study these images to interpret which genes are likely involved, how they change the cellular phenotype, and how these genes modulate their function depending on the surrounding microenvironment.

In collaboration with Dr. Timothy Chan at Memorial Sloan Kettering Cancer Center (MSKCC) and Dr. Molly Hammell (CSHL), we are investigating the gene activity in various human brain tumors, including low-grade glioma (LGG). The causative mutation in LGG is isocitrate dehydrogenase 1 (IDH1), which drives hypermethylation of the genome through indirect inhibition of DNA and histone demethylases. To quantify the selection pressure on LGG, we are profiling genetic mutations, genome methylation, and transcriptome in situ. Recent studies, including from Dr. Chan’s laboratory, have established LGG as a gold standard in studying the correlation among clinical history, histology, genetic profiles, and clinical biomarkers. Our goal is to test the utility of FISSEQ in obtaining clinical biomarkers of early brain cancer directly from tissue sections. Then, we will test their efficacy in identifying known and unknown glioma subtypes in clinical specimens. This novel approach has the potential to transform how cancer researchers study the genetic cause of abnormal features they see under the microscope. Using FISSEQ, they will literally see the genetic sequences in the microscope image.

More fundamentally, we are investigating metabolic reprogramming and subcellular compartmental-
Cancer: Genetics

The Genetic Basis of Pattern Formation in Limb Development
E. Rozhkova [in collaboration with J. Sharpe, CRG, Barcelona]

We are investigating the classic assumption that the genetically encoded diffusion rate of morphogen is the driving force behind cellular self-organization and tissue patterning. We hypothesize that local cellular interpretation of morphogen signaling could vary depending on the environmental context and that physical forces and cell cycle regulation further modulate developmental signaling for robust patterning.

In collaboration with Dr. James Sharpe (CRG, Barcelona), we are quantifying the gene activity in early mouse limb development using FISSEQ and laser-capture microdissection RNA-seq. Our goal is to visualize the periodic gene activity involved in mouse digit patterning in response to Wnt and BMP4. This work enables us to identify additional genes involved in tissue patterning beyond those that are previously characterized by forward and reverse genetic approaches. Moreover, our approach is ideal for discovering the spatial enrichment of poorly annotated genes, non-coding RNAs, or low-abundance genes previously ignored. Finally, we will be using the spatial frequency of gene expression in limb development to study chromatin biology and enhancer regulation by developing additional methods such as ChIP-seq (chromatin immunoprecipitation sequencing) and ATAC-seq (assay for transposase-accessible chromatin with high-throughput sequencing) in situ.

We are also investigating whether epithelial morphogenesis in human embryonic stem (ES) cell development is dependent on Wnt- and DKK-dependent Turing mechanisms. To quantify the effect of cell cycle and morphogen concentrations, we are using live cell cycle indicators (FUCCI) and optogenetic morphogen signaling, and we are using FISSEQ to profile how cells interpret morphogenic cues in a context-dependent manner. Our goal is to quantify spatial oscillation in gene expression that can generate scalable tissue patterns in spite of the highly noisy and/or unpredictable cellular environment in vivo. Cell lineage tracing during ES cell development is essential for understanding how individual cells decide their fate during early morphogenesis. In collaboration with Dr. Michalis Averof (Institut de Genomique Fonctionnelle de Lyon [IGFL]) and Dr. Max Telford (University College London [UCL]), we are developing a cell lineage–tracing tool using FISSEQ and applying it to study Drosophila embryogenesis. We believe that it will be possible to trace the lineage of embryonic, intestinal, and cancer stem cells in vivo using genetic barcode sequencing in situ in the near future.

Technology Development for Next-Generation Cell Biology
E. Rozhkova, D. Ghosh

Although FISSEQ can detect gene activity in specific tissue regions transcriptome-wide, one main drawback is its lack of sensitivity for targeted RNA sequencing in situ. Over the last year, we have developed a novel method to transform fluorescent in situ hybridization (FISH) into a highly sensitive and direct
RNA sequencing method. Furthermore, we have introduced key technological innovations to eliminate the need for high-resolution imaging for accurate molecular quantification in situ. This will enable one to paint various genetic elements in the genome, including miRNA, noncoding RNA, and T-cell receptors, in situ to investigate their role in development and in cancer and other diseases. Our method also allows multiple genetic perturbations and sequencing barcodes associated with reporters, cells, or Cas9/shRNA for pooled functional analysis in vivo and in situ. Our ultimate aim is to enable a highly multiplexed and single cell–based experimental biology platform using in situ sequencing technologies, and we hope that it will have a broad impact on how cell and developmental biology are done in the future.
Research in our lab is focused on determining the genetic/epigenetic basis of neurodevelopmental syndromes and cancer. We have discovered new genes that affect these conditions, revealed mechanisms for how the encoded proteins normally work, and determined how defects in these processes lead to disease. These findings have had a major impact in the scientific community, and have affected how clinicians analyze and treat patients with these syndromes.

Major discoveries:
- Identifying p63 as a gene affecting development, aging, and cancer
- Defining the genetic basis of autism
- Discovering CHD5 as a gene that prevents cancer
- Determining that Chd5 loss causes male infertility

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

We discovered p63, a gene that is related to p53—a well-studied gene known to be defective in more than half of all human cancers. Although it was similar to p53, it was not at all clear how p63 worked. We found that a lack of p63 leads to aging, as p63 is needed for stem cell renewal, and a lack of p63 causes 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. Our work showing that TAp63 inhibits tumor growth, even when p53 is absent, was surprising: It had always been believed that p53 was absolutely essential for inhibiting cancer. Instead, we found that TAp63 can do the job alone.

When we first identified p63 we discovered that it was needed for development: Its loss causes malformations of the limbs, skin, and palate. This finding led others to interrogate p63, and to reveal that its mutation causes seven different human syndromes involving birth defects affecting the limbs, skin, and palate. By generating mouse models for one of these syndromes, called EEC (ectrodactyly, ectodermal dysplasia, clefting) syndrome, revealing new insight into why some children with EEC syndrome have symptoms that are very severe and even life threatening, other children with EEC—even those in the same family with the same p63 mutation—have symptoms that are barely noticeable. We are currently working to understand how p63 regulates stem cell biology and how its perturbation leads to cancer.

**Discovering CHD5 as a New Cancer-Preventing Gene**

We 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, we generated 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—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. Chromosome engineering proved so powerful for discovering the 1p36-encoded gene that had challenged the cancer community for more than three decades that we also used it for studying neurodevelopmental syndromes, including schizophrenia.
and autism. This technology was essential for several collaborative studies published during the past year, including those focused on copy number variations responsible for cancer (Liu et al. 2016) and autism (Tian et al. 2015; Brunner et al. 2015).

We continue to focus on defining the role of CHD5 in chromatin dynamics and deciphering how dysregulation of CHD5 and the pathways it regulates leads to disease. We found that Chd5 uses its plant homeodomains to bind histone 3, and that this interaction is essential for tumor suppression. Our work paved the way for further discoveries, and 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 patients with high levels of CHD5 have much better overall survival than those with low levels. We found that Chd5 is essential for packaging DNA, and that loss of Chd5 causes improperly packaged DNA that is prone to DNA damage. Intriguingly, Chd5’s absence is particularly important during the process of sperm maturation—an event in which the DNA is first unpackaged and then repackaged using an elaborate series of steps—and that deficiency of Chd5 causes male infertility. We discovered that Chd5 is expressed highly in neurons, and within the last year we found that Chd5 plays a pivotal role in the brain, suggesting that inappropriate DNA packaging contributes to neurodevelopmental syndromes such as autism. We are currently delving deeper into the mechanisms whereby Chd5-mediated regulation of chromatin affects gene expression cascades that regulate neuronal stem cells, and how deregulation of this process sets the stage for neurodevelopmental syndromes and cancer.

PUBLICATIONS


In Press

CANCER GENOMICS

S. Powers  F. Celeste  M. Rao
J. Li  X. Zhao
A. Mofunanya

Our focus remains on using the tools of genetics and related disciplines (e.g., genomics, systems biology, microevolution) to study cancer.

Functional Genomic Screening for Drivers and Therapeutic Targets

A. Mofunanya, J. Li

Much of our work is funded by the National Cancer Institute (NCI) to work individually and collaboratively (CTD2; https://ocg.cancer.gov/programs/ctd2) to use cancer genome data for the discovery and development of human cancer therapeutics. Some of the best cancer drug targets are the structurally altered or overexpressed proteins that are responsible for driving the oncogenic phenotype. We used pooled screening and next-generation sequencing to examine the ability of a large set of overexpressed genes in human breast cancer to drive tumorigenic properties and tumorigenesis in model systems. In addition to detecting known drivers, such as WWTR1/TAZ, we discovered that CEACAM5, a well-known tumor marker thought to be a passive passenger, plays an active role in tumorigenesis, as does SCUBE3, a secreted protein that we are pursuing as a therapeutic target. Genetic analysis indicates that SCUBE3 plays an important role in the ongoing tumorigenic properties of several breast cancer cell lines.

MAPAC: A Modeling-Based Approach to Analyze Clinical and Phenotypic Effects of Copy Number Alterations Using Large-Scale Cancer Genome Data Sets

J. Li [in collaboration with J. Pollack and R. Tibshirani, Stanford; W. Zhao and G. Mills, MD Anderson Cancer Center]

Copy number alterations (CNAs) play a key role in the pathogenesis of human cancers, yet little is known about the phenotypic impact of most specific alterations. The storehouse of information present in large-scale integrated cancer genome data sets should help solve this problem. Prior analytical efforts have relied on gene-by-gene analysis, which obscures the multigenic nature of CNAs and their frequent co-occurrence with other distal CNAs. Here we have developed an alternative that focuses on CNAs themselves through a computational approach that models the copy number profile of each individual tumor. We applied this modeling approach to two large breast cancer data sets (METABRIC and TCGA) and used it to tease apart the effects of two frequently co-occurring CNAs on chromosome 11, CCND1 amplification, and the more frequently occurring heterozygous loss of 11q14-q25. We found that loss of 11q14-q25 drives poor clinical outcome in luminal tumors and that CCND1 amplification in this context is a passenger event. Paradoxically, although YAP1 is located within this deletion, the amount of unphosphorylated, activated Yap1 protein itself is elevated in deleted tumors, and this may underlie the association with poor outcome. Additionally, we determined that PTEN loss was linked to increased Akt phosphorylation and poor prognosis in basal-subtype tumors. Overall, we observed extensive cis effects of CNAs on messenger RNA (mRNA) abundance and corresponding protein levels. We have also shown that any new individual tumor copy number profile can be matched to the modeled data, opening the door to more extensive use of copy number profiling in patient prognosis and treatment.

Effectors and Potential Targets Selectively Up-Regulated in Human KRAS-Mutant Lung Adenocarcinomas

J. Li [in collaboration with R. Sordella, CSHL]

Genetic and proteomic analysis of human tumor samples can provide an important complement to information obtained from model systems. Here, we examined
Research

protein and gene expression from the Cancer Genome and Proteome Atlases (TCGA and TCPA) to characterize proteins and protein-coding genes that are selectively up-regulated in KRAS-mutant lung adenocarcinomas. Phosphoprotein activation of several Raf/MAPK signaling components was considerably stronger in KRAS mutants than any other group of tumors. However, both KRAS mutants and tumors with other activating mutations in the Raf/MAPK pathway showed comparable activation of mTOR (mechanistic target of rapamycin). Co-occurring mutations in KRAS mutants were associated with differential activation of PDK1 and PKC-α. Genes showing strong activation in RNA-sequencing (RNA-seq) data included negative regulators of Raf/MAPK signaling, along with potential oncogenic effectors, including activators of Rac and Rho proteins and the receptor protein-tyrosine phosphatase gene PTPRE. These results corroborate Raf/MAPK signaling as an important therapeutic target in KRAS-mutant lung adenocarcinomas and pinpoint new potential targets.

Accurately Modeling Multigenic and Large Copy Number Alterations in Human Breast Cancer
M. Rao

Multigenic and large CNAs are a dominant feature of human breast cancer, but there is still a total lack of experimentation to determine specific effects. We are using CRISPR–Cas9 and chromosomal engineering technology to test the oncogenic effects of both large chromosomal duplications and heterozygous loss, using the MCF10A preneoplastic cell and other so-called normal cells as models. Additionally, we are using CRISPR–Cas9 genomic engineering to introduce an amplifiable DHFR gene into multiple genomic sites to examine the effects of localized amplification of different regions on tumorigenic properties.

Genetic Interaction Sequencing
X. Zhao [in collaboration with S. Levy, Stony Brook Laufer Center]

We are developing a platform that will be able to generate and assay millions of combinations of CRISPR–Cas9 single-guide RNAs (sgRNAs) or other genetic perturbagens. Doing so will clear the way for systematic exploration of genetic interactions in mammalian cells, but success depends on several high-throughput steps, including a dependable gene knock out or knockdown method, a method to deliver two gene knockouts/knockdowns into the same cell and to monitor which cells receive which combinations, and a reliable assay to measure relative fitness. Our major enabling technology for development of a high-throughput system for mammalian cells is the tandem-integration landing pad that allows two plasmids to be inserted next to each other at a neutral location of the genome. Each plasmid contains a DNA barcode that uniquely identifies the associated genetic perturbagen (e.g., sgRNAs). When both plasmids are integrated into the genome, the two barcodes are in close enough proximity to be sequenced together by paired-end amplicon sequencing. We have established this methodology in yeast and have shown that it can generate a library of more than 10^6 double-barcoded cells via pooled sequential plasmid transformation and integration. The fitness of large double-barcode libraries can then be measured using the fit-sequencing (fit-seq) approach that we pioneered: Pooled growth and double barcode amplicon sequencing over several time points accurately measures the relative fitness of each double-barcoded cell in the pool. In yeast, genetic interaction sequencing (GiSeq) promises to be a cheaper and higher-throughput alternative to the commonly used synthetic–genetic array technology. In mammalian cells, GiSeq promises to be a major leap forward over existing technologies: Not only will genome-scale interaction libraries become practical, but negligible work will be needed to repeat a screen in different cells or different conditions.

Creating an Interdisciplinary Team to Study Evolutionary Dynamics in Cancer
F. Celeste [in collaboration with M. Egeblad, CSHL; E. Coutsias, S. Levy, F. Talos, and S. Wu, Stony Brook University]

Recent analysis with deep sequencing has shown that human cancers are continually evolving and that this property is what defeats many targeted therapies, as well as other systemic treatments such as chemotherapy. In general, the evolutionary dynamics of cancer have not been studied directly and there are likely several fundamental aspects to cancer that remain unknown as a result. To address this, our group has been collaborating with others here at Cold Spring Harbor Laboratory and Stony Brook to develop a robust system
based on the high-resolution lineage tracking methodology established in yeast by Dr. Sasha Levy (Levy et al. 2015. Nature 519: 181). Using this methodology, Dr. Levy discovered that the early phases of adaptive evolution in yeast to limiting glucose involve hundreds of small adaptive alterations, and that it takes considerable time before mutations with strong selective advantages (e.g., mTOR, RAS) dominate the landscape. If one draws an analogy to cancer, all of the genetic changes with which we are familiar (e.g., RAS, TP53 mutations) are likely to represent the latter changes seen in yeast, which implies that the initial critical steps of cancer initiation or early development of resistance are completely undescribed at the molecular level.

To create an interdisciplinary team that has the depth in tools and approaches to tackle this problem, we are working with Dr. Flaminia Talos on integrating the high-resolution lineage tracking system with the “Confetti Mouse” lineage-tracing methodology she has used to study prostate cancer evolution and progression in vivo. We are also working with Dr. Mikala Egeblad to integrate her intravital imaging of chemotherapeutic response and the use of Confetti technology to study the development of resistance in vivo. Additionally, we are working with Dr. Song Wu and Dr. Evangelos Coutsias to create robust computational and theoretical pipelines to analyze our lineage tracking data. Our goal is to create new fundamental knowledge about malignant cell evolutionary dynamics and open up a new approach to therapeutics based on deeper understanding of the evolutionary forces that drive resistance.

PUBLICATIONS


In Press


ANEUPLOIDY AND GENE DOSAGE IMBALANCES IN CANCER

J. Sheltzer  C. Giuliano  A. Lin  N. Sayles

The Sheltzer Lab was founded in August 2015. We are working to combine in silico, in vitro, and in vivo approaches to address several questions in cancer genetics. In particular, we are deeply interested in the consequences of genomic imbalances on tumor development and progression. Genomic imbalances are found in nearly every cancer cell and can alter the dosage of hundreds of genes at once. Approximately 90% of solid tumors display whole-chromosome aneuploidy, whereas many tumors with diploid karyotypes nonetheless harbor segmental or arm-length aneuploidies that also result in significant gene copy number alterations. The prevalence of aneuploidy in cancer—first noted more than 100 years ago—has led to a widespread belief that genomic imbalances play a crucial role in tumor development. Indeed, in the early 20th century, Theodor Boveri speculated that abnormal karyotypes altered the balance between pro- and antiproliferative cellular signals and were therefore sufficient to induce transformation. “Boveri’s hypothesis” has motivated nearly a century of research into the origins and consequences of aneuploidy, but the precise relationship between abnormal karyotypes and tumorigenesis remains unclear.

**Single-Chromosome Aneuploidy Commonly Functions as a Tumor Suppressor**

To test the link between aneuploidy and cancer, we transduced congenic euploid and trisomic fibroblasts with 14 different oncogenes or oncogene combinations, thereby creating matched cancer cell lines that differ only in karyotype. Surprisingly, nearly all aneuploid cell lines divided slowly in vitro, formed few colonies in soft agar, and grew poorly as xenografts, relative to matched euploid lines. Similar results were obtained when comparing a near-diploid human colorectal cancer cell line with derivatives of that line that harbored extra chromosomes. Only a few aneuploid lines grew at close to wild-type levels, and no aneuploid line exhibited greater tumorigenic capabilities than its euploid counterpart. These results demonstrate that rather than promoting tumorigenesis, aneuploidy can very often function as a tumor suppressor. Moreover, our results suggest one potential way that cancers can overcome the tumor suppressive effects of aneuploidy: Upon prolonged culture in vitro or in vivo, cell lines with simple aneuploidies developed recurrent chromosomal aberrations that were absent from their euploid counterparts and that were associated with enhanced growth. Thus, the genome-destabilizing effects of single-chromosome aneuploidy may facilitate the evolution of balanced, high-complexity karyotypes that are frequently found in advanced malignancies.

**New Models of Aneuploidy**

Thus far, our ability to study aneuploidy has predominantly been limited to cells with single-chromosome gains grown in vitro. We are developing several approaches that will allow us to interrogate different types of aneuploidy in distinct cellular contexts. First, through the use of CRISPR/Cas9, we are generating mouse embryonic stem cells that harbor segmental deletions and duplications. Mice that harbor these alterations will be studied to identify the consequences of segmental genomic imbalances in vivo. Second, we are using the long noncoding RNA Xist to generate cellular models of chromosome loss. When Xist is integrated into an autosome, transcriptional activation of Xist is sufficient to block gene expression from that chromosome, thereby mimicking a monosomic state. Cells with and without Xist will provide a genetic system to study the acute effects of chromosome loss on cellular physiology and transformation.

**Discovery and Characterization of Genes Affecting Survival Time in Cancer**

In addition to our investigation of aneuploidy, we are working to uncover the molecular differences between benign and aggressive tumors. In human patients, certain...
To uncover new cancer genes and to increase our understanding of the molecular differences between fatal and nonfatal tumors, we are analyzing data derived from cancer survival studies. In these studies, investigators profile tissue from tumors surgically excised from patients and then link that data with clinical information on the patient’s tumor, treatment regime, and the length of time until death and/or another adverse event occurred. Tumors included in these studies are typically matched for stage, grade, and treatment, and only pure samples that yield high-quality RNA (or other biological molecules) are analyzed. Genes whose expression consistently correlates with increased or decreased survival time in cancer may represent novel oncogenes, tumor suppressors, or mediators of the metastatic state. We are therefore deploying CRISPR technology to modulate the expression of these genes in primary cells and in cancer cells to establish molecular links between these genes and cancer prognosis.
CANCER AND HUMAN GENETICS

M. Wigler  R. Aboukhalil  J. Kendall  S. Mukhopadhyay  J. Troge
P. Andrews  B. Ma  M. Ronemus  B. Yamrom
T. Forcier  S. Marks  J. Rosenbaum  C. Yoon
I. Hakker  J. McIndoo  A. Stepansky

Our laboratory works in three areas: cancer, genomics, and autism. Genomics is the bridge between our research areas. For cancer, we attempt to use genomic analysis of single cells to improve clinical understanding of cancer: risk assessment, detection, and therapeutic response. For genomics, we develop informatics and wet-bench techniques that allow greater access to genomic variation and organization. For autism, we seek to understand the genetic causation of the disease through sequence analysis of members of affected families. We have major intramural collaborations with members of the Simons Center for Quantitative Biology—namely, Dan Levy, Ivan Iossifov, Alex Krasnitz, and Partha Mitra. Some of this work is in collaboration with the New York Genome Center, the Simons Foundation, and other collaborators, generally in the New York area.

CANCER

Single-Cell Genomic Analysis of Prostate Cancer

J. Alexander, R. Aboukhalil, and J. Kendall [in collaboration with A. Krasnitz, CSHL]

During previous years, we showed methods for the genomic analysis of single cancer cells and their utility in illuminating the clonal heterogeneity of cancers and the derivation of phylogenies (Navin et al., Genome Res 20: 68 [2010]; Navin et al., Nature 472: 90 [2011]). We have applied these methods to prostate biopsies comparing single-cell genomics to histopathology for detecting cancer and risk assessment. From the order of 10 cases, examining hundreds of cells from each patient, we have shown the essential equivalence and potential superiority of our method over histopathology, as it yields information about clonal substructure, degree of genome instability, and clonal spread. This work was made possible by the building of a “single-cell viewer” that integrates genomic data with anatomy and gene-based information. In the future, we will improve the viewer and develop newer single-cell methods that “see” more and are cheaper. This work is now being written for publication.

Improved Single-Cell DNA Methods

Z. Wang, J. Alexander, A. Stepansky, and J. Kendall

We have made and published improvements in single-cell DNA analysis; yet two factors still limit its application in a clinical setting: It is expensive, and it yields only copy number profiles of individual cells. Hence, we have developed a new method based on the idea of representations: cleavage of single-cell DNA, ligation to adaptors with tags, and amplification. The method uses less expensive reagents, and earlier pooling also makes it less expensive and technically more manageable and, in fact, adaptable to a mega-array format. Because the DNA amplified derives from a representation, we predict that the method will also detect cancer-specific single-nucleotide variants (SNVs), which will be of use in determining genomic stability, improving phylogeny, and deriving cancer signatures useful for other projects, as described below. This work is now being written for publication.

Sensitive Detection of Cancer Signatures

Z. Wang and J. Kendall [in collaboration with D. Levy, CSHL]

We have developed methods to correct most sequence errors arising from polymerase chain reaction (PCR) and platform artifacts. The technique is to add varietal tags (Hicks et al., US patent publication US 2014 and US 0065609 [2014]; Kiuvoja et al., Nat Methods 9: 72 [2012]) to template molecules before any copying, so that sequence divergence from the same template can be filtered away. This method should find use in the extremely sensitive detection of cancer signatures,
which are sequences found in the cancer but not in
the patient’s normal somatic tissues. This work is now
being written for publication. We are preparing to use
the technique in several critical clinical contexts: mea-
suring residual disease (to determine when therapy has
been successful) and monitoring the early detection of
reurrence. In the event that we can discover through
single-cell analysis subclonal signatures, we may be
able to rapidly monitor which subclonal populations
respond to a given therapy, setting up a rapid cycle
of treatment and response—ultimately leading to em-
pirical selection of the optimum combinations of che-
motherapies. All these methods depend on finding at
least traces of cancer DNA in the circulatory system
that reflect its physiological state: load and death. The
physiology is not yet worked out.

Spatial Genomics
A. Stepansky and M. Ronemus [in collaboration with
P. Mitra, CSHL]

If we are to truly grasp cancer at a genomic level while
recognizing that cancers are heterogeneous, it is nec-
essary to develop a spatial configuration of the sub-
populations with their signatures. In part, this is to be
comprehensive, so that all subpopulations are targeted
by therapy and, in part, so as to study the pattern of
subclonal spread, which may offer clues of risk and
lessons in cancer pathophysiology. For this purpose,
we have joined forces with Partha Mitra’s laboratory.
His group has expertise in reconstructing anatomy
from cryo-sectioning and microscopic imaging—just
the skill set needed to develop spatial cancer genom-
ics. Preliminary design requires serial sectioning with
“punches,” and preliminary experiments indicate ex-
cellent recovery of nucleic acid from punches.

GENOMICS
Phasing Heterozygosity
Z. Wang and P. Andrews [in collaboration with D. Levy
and P. Mitra, CSHL]

In anticipation of its need for haplotype-specific ge-
nome assembly and the detection of loss of heterozy-
gosity in cancer, we have initiated a program to deter-
mine the phasing of heterozygous sites in individual
genomes. The method is both amusing and surprising:
Prepare cells in a frozen block, then make fine cuts in
a microtome, flow sort the subnuclear fragments, and
apply methods for single-cell DNA amplification. It
works: Many nuclei are cleaved, leaving large single
chromosomal blocks intact. From the correlation of
heterozygous SNPs we are able to reconstruct the in-
dividual’s phased loci. This method also probably has
general applicability to long-range assembly, exceed-
ing the possible linkages of any existing method. We
call the method “HaHa,” for haplotyping through
halving.

Bisulfite Template Mutagenesis
J. Kendall [in collaboration with D. Levy and V. Kumar,
CSHL]

Last year, we published a theory paper on the assets of
random template mutagenesis in problems of count-
ing and assembly of nucleic acid sequences (Levy and
Wigler, Proc Natl Acad Sci 111: E4632 [2014]). This
year we showed that the method works using partial
bisulfite mutagenesis. This is a method used to ob-
serve cytosine methylation, in which case the user
seeks to saturate the conversion of unmethylated Cs
to Us. In our case, we seek partial conversion and con-
trol the reaction by reduced temperature and time.
It works beautifully, and exactly as theory predicts.
The conversion is site-independent. We obtain accu-
rate counting, as precise as can be achieved with bi-
nomial sampling, and can assemble long transcripts.
This work is now being written for publication. We
are contemplating using the method, together with
HaHa, to attempt full haplotype assembly of mem-
bers of a family trio. One purpose will be to assess
the types of de novo structural rearrangements that
we cannot presently observe by short read sequencing.
Another purpose is to perform accurate tissue-specific
and single-cell transcriptome characterization.

Single-Cell RNA
Z. Wang and J. Kendall [in collaboration with D. Levy,
CSHL]

In separate collaborations with Dan Geschwind at
UCLA and Josh Huang of CSHL, we are attempt-
ing to develop robust methods for profiling the trans-
scriptome of single neuronal cells. Present methods
are adequate to draw some conclusions, but they are
not robust in our hands. The problem is low amounts
of starting material and, hence, “jack-potting”; the
emergence of rapidly amplifying copy DNA (cDNA) fragments. We are exploring bisulfite mutagenesis as a more robust method that, in combination with cell tagging, would allow early pooling and, through assembly, provide data about single-cell splicing patterns.

SMASH

Last year, we developed a method for copy number profiling that uses sparse sequencing. We fragment the DNA into small pieces, then ligate them, and prepare libraries. The size of the fragments, between 30 and 40 nucleotides, is long enough to give unique mappings into the genome, yet so small that we can get on the order of two to three mappings per read and twice that number from read pairs. This is the most efficient mode for obtaining copy number profiles from current day sequencing platforms. In the past year, we made a major improvement in technique, utilizing NEB fragmentase to create fragments, thus streamlining the protocol. The method should find utility in family genetic counseling by providing an affordable high-resolution search procedure for de novo copy number events common in pediatric disorders such as autism and cardiac developmental anomalies. This work has just been accepted for publication.

MUMdex
P. Andrews and J. Kendall [in collaboration with D. Levy and I. Iossifov, CSHL]

We have been dissatisfied with existing software tools for detecting structural rearrangements in genomic DNA and so built our own based on a new (but, retrospectively, rather obvious) idea that we call the “bridge invariant.” MUMdex is a software package for genome data analysis based on maximal unique matches (MUMs). A MUM between two sequences is defined as an exact match subsequence that exists only once in each sequence and is not part of any longer exact match. A MUM is a good starting point for inference about sample genome structure because of its unique reference coordinate.

The bridge invariant is created by every rearrangement of the genome. Imagine two different genomic rearrangements: a deletion (case 1) and one end of an inversion (case 2). In both cases, an event joins two pieces of the genome not previously adjacent, creating a bridge. Each piece has coordinates in a reference genome, and these coordinates can be extended locally to adjacent base pairs even beyond the bridge. There are two such coordinate extensions at every base in the region, and they conflict. In the first case, the difference between the two coordinates is a nonzero constant. In the second case, the sum of the coordinates for any position is a nonzero constant. We call the constant associated with a recombination event the bridge invariant because it bridges two views of the recombined site, and it can be computed from any sequence read that spans the join with enough sequence to establish MUMs on either side of the event. The MUMs, with their unique mapping to the genome, impart the coordinate extensions to the reads. The properly computed invariant will not depend on the strand read, the start position of the read, read length, or base calling errors. We use population databases to remove common variants that arise from defects in the reference genome. MUMdex has proven utility for detecting rearrangements in cancer (compared with the normal) and de novo events in children (compared with their parents). This work is now being written for publication. We are working on packages to make the software widely available, and have established its infrastructure, as well, at the New York Genome Center.

High-Throughput Single-Cell Processing
Z. Wang [in collaboration with S. Li and D. Levy, CSHL]

In collaboration with Eric Brouzes at Stony Brook University, an expert in microfluidics, we have been exploring methods for inexpensive high-throughput analysis of single cells. Our present ideas are based on combinatorial tagging, varietal tagging, and oil immersion delivery systems. Although still in its early stages, we are optimistic that new ideas of combinatorial tagging will enable us to reach goals of very low cost single-cell analysis, and preliminary experiments are encouraging. We should have more details about methods to report next year.

AUTISM
The goals of our autism research have been driven by discovering the vulnerable genetic targets of causative
mutation, and constructing quantitative models to explain its epidemiology as far as is possible by genetics. We have focused mainly on rare variants, occurring de novo or transmitted, and, in collaboration with Kenny Ye of Albert Einstein College of Medicine, we have also explored whether there is any demonstrable role for common variants.

Genetic Modeling
Swagatam Mukhopadhyay, Mike Ronemus, and Boris Yamrom [in collaboration with K. Ye, I. Iossifov, and D. Levy, CSHL]

In 2008, we proposed what we called the unified genetic model for autism, in which much of simplex autism is caused by de novo mutation (Sebat et al., Science 316: 445 [2007]; Zhao et al., Proc Natl Acad Sci 104: 12831 [2007]; Iossifov et al., Nature 515: 216 [2014]; Ronemus et al., Nat Rev Genet 15: 133 [2014]), and females can be the preferred asymptomatic carriers of new damaging mutation because they are intrinsically refractory to the disorder. A formal mathematical analysis of the model, which fits epidemiological data very well, was published in the bioRxiv. However, we have expanded the model to a two hit model with rare strong variants (mostly occurring de novo), and less rare and weaker variants (mainly transmitted). There are parameter settings that fit the epidemiological data, as well as the one-class model, and the two-hit model has advantages: It explains better that lower severity children are almost all male. We are in a much delayed process of writing up a full manuscript of the two-class model.

Vulnerable Genes as De Novo and Transmitted Targets

We anticipated and then showed that the gene targets of de novo disruptive mutation in children also show evidence of being highly conserved in the human gene pool. We reversed that inference, and used gene vulnerability, as evidenced by low variance in the human gene pool, to produce a list of the best 80 candidate autism target genes. We then showed that rare disruptive variants in vulnerable genes are more likely to be transmitted to affected male offspring than to unaffected male offspring, and typically originate from the mother. These findings are in keeping with the tenets of the unified theory of genetic causation. This work was recently published.

Transmission of Common Variants
This work was done in collaboration with K. Ye and I. Iossifov (CSHL)

Several recent studies claimed that common variants collectively have a significant role in autism spectrum disorder (ASD). Such claims are based on a liability threshold model, under which a set of genetic variants and environmental factors contribute to a liability score additively, and those individuals whose liability scores are above a threshold get the disease and those whose liability scores are below the threshold model are disease-free.

We hypothesized that if the common variants collectively contribute significantly to a disease, given a discordant sib pair, one affected and one unaffected, other unrelated affecteds are likely to share more common variants than unaffected sibs from two unrelated families. Based on this prediction, we developed a statistic, $A^2DS$, to capture the differences of genetic relationship among affected individuals to discordant sibships. Our prediction is found correct in simulations of the liability threshold model. Moreover, by permuting disease status of the sibpairs, the statistical significance of any contribution from common variants can be tested using $A^2DS$ as the test statistic. Given 2000 discordant sib pairs, our statistic has very good power to detect a sizable contribution from common genetic variants.

However, we did not find statistical significance when we applied our method on 2591 Simons Simplex Collection (SSC) families genotyped using SNP arrays and 1865 families genotyped by whole-exome sequencing. This leads us to believe that the previously reported contribution from common variants to ASD was overstated and perhaps nonexistent. The overestimation of heritability in those studies might be caused by some systematic biases in genotyping between the cases and controls and/or subtle differences in population ethnic background between the cases and controls. We are in the process of writing up our results, combined with a critique of the abuses by others of the foundational paper by Yang et al. (Am J Hum Genet 88: 76 [2011]).
Whole-Genome Sequencing
This work was done in collaboration with I. Iossifov and his group (CSHL)

Our previous autism work was performed by microarray and, then, whole-exome sequencing. We have now begun analysis of whole-genome sequencing of two large collections: the SSC and the Autism Genetic Resource Exchange (AGRE) multiplex collection. It is too early to state conclusions, as we have mainly been establishing pipeline. Hopefully, the outcome will be: discovery of new structural variants and noncoding variants, validation of existing candidates, and information from which we can build a stronger model for transmission genetics.

PUBLICATIONS

In Press
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.

Douglas Fearon’s laboratory studies the interaction between cancer and the immune system. Their underlying premise is that the tumor microenvironment is immune suppressive because cancer cells elicit responses characteristic of wound healing and tissue regeneration. This approach has led to the finding that activated fibroblasts in the tumor stroma mediate immune suppression in several mouse models of cancer, including the autochthonous model of pancreatic ductal adenocarcinoma of the Tuveson lab. Our understanding of the basis of immune suppression is evolving, but we know that it involves the production of the chemokine CXCL12 by the fibroblastic stromal cells, binding of this CXCL12 by pancreatic cancer cells, and exclusion of T cells from the vicinity of the cancer cells. T-cell exclusion, which also occurs in several types of human adenocarcinomas, causes antagonists of T-cell checkpoints to be ineffective, despite the presence of cancer-specific CD8+ T cells. This immune suppression is interrupted by administering AMD3100, an inhibitor of CXCR4, the receptor for CXCL12, which leads to the rapid accumulation of T cells among cancer cells, thereby uncovering the efficacy of anti-PD-L1 and eliminating cancer cells. Because human pancreatic cancer has certain immunological characteristics of the mouse model, a phase 1 clinical trial of AMD3100 in patients with pancreatic cancer was initiated in 2015. Some of our next steps are to determine the biological process that causes cancer cells to express nonmutated, shared antigens and the means by which dormant metastases escape immune elimination.

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 (H₂S) under conditions of endoplasmic reticulum (ER) stress that are linked to protein folding–related pathologies, such as Parkinson’s and Alzheimer’s. In addition, they have generated recombinant antibodies that 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.

Dr. Tuveson serves as Director of the Lustgarten Foundation Pancreatic Research Laboratory at CSHL and as Director of Research for the Lustgarten Foundation.

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  J. Albregues  E. Bruzas  M. Fein  V. Kuettner  J. Park  M. Terp
A. Almeida  J. Cappellani  Y. Jia  L. Maiorino  M. Shields  R. Wysocki

Solid tumors are aberrant tissues. Like organs, solid tumors 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 confocal 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.

The Influence of the Tumor Microenvironment on Cancer Progression Is Different in Different Cancer Subtypes

The stroma of breast cancer can promote the disease’s progression, but whether its composition and functions are shared among different subtypes is poorly explored. We compared stromal components of a luminal (MMTV-Neu) and a triple-negative/basal-like (C3[1]-Simian Virus [SV]40 Large T antigen [Tag]) genetically engineered breast cancer mouse model. The types of cytokines and the extent of their expression were very different in the two types of breast cancer, as was the extent of innate immune cell infiltration; however, both models showed infiltration of innate immune cells that expressed matrix metalloproteinase 9 (MMP9), an extracellular protease linked to the progression of many types of cancer. By intercrossing with Mmp9 null mice, we found that absence of MMP9 delayed tumor onset in the triple-negative model, but had no effect on tumor onset in the luminal model. We discovered that this correlated with the presence of insulin-like growth factor binding protein 1 (IGFBP-1), which is a known substrate for MMP9. IGFBP-1 binds and antagonizes insulin-like growth factors (IGFs), preventing them from activating their receptors to promote cell proliferation and survival. Tumors from C3(1)-Tag;Mmp9−/− mice had reduced IGF-1 receptor phosphorylation, consistent with slower tumor onset. This suggests that although IGFBP-1 can inhibit cancer progression, when MMP9 is present, it degrades these IGFBPs and releases the IGFs. The release of the IGFs then accelerates cancer progression. Consistent with results from our mouse data, gene expression analysis of human breast tumors showed that high expression of IGFBP messenger RNA (mRNA) was strongly correlated with good prognosis, but not when MMP9 mRNA was also highly expressed. The study’s results may explain why previous clinical trials using MMP inhibitors have failed, as patients were never enrolled based on expression of MMPs or their substrates.

The Influence of the Tumor Microenvironment on Response to Therapy

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 pathways proposed for the origin of the therapy-resistant cancer cells: (1) from subpopulations of cells already present within the tumor, (2) from cells with stem cell–like properties, or (3) through mutations or epigenetic changes. Understanding how each of these mechanisms drives resistance is critical to reducing recurrence.

We previously used intravital imaging to show that monocytes are recruited to tumors after chemotherapeutic treatment with doxorubicin. We further...
determined that the infiltration of these monocytes contributed to chemoresistance. To identify how such microenvironmental changes contribute to the selection of chemoresistant breast cancer, we are using a combination of lineage tracing, intravitral imaging, and microgenomics approaches. The ultimate goal is to shed light on whether the acquisition of mutations that cause therapy resistance can be prevented by limiting factors in the microenvironment that lead to accelerated mutation rates.

We have developed a lineage-tracing model consisting of primary cancer cells, isolated from MMTV-PyMT mice crossed with so-called “Confetti” mice. Cancer cells from these MMTV-PyMT;Confetti2 mice are infected in vitro using an adeno-cre construct, resulting in random expression of any one of 10 different combinations of fluorescent proteins in clones of cancer cells. These PyMT;Confetti2 cells are then injected back into syngeneic mice, where they form tumors. We performed imaging of tumors of either untreated mice or mice treated with chemotherapy with cisplatin. Tumors in untreated mice retain a random distribution of differently colored cells, representing the lack of clonal selection in the absence of treatment. In contrast, on chemotherapy with cisplatin, clonal selection was very evident as shown by the outgrowth of large colonies of identically colored cancer cells.

### Cancer Cells Orchestrate Escape from the Adaptive Immune Response

**M. Fein, A. Almeida, J. Cappellani**

The immune system is very efficient at eliminating pathogens that can cause harm to the organism. The immune system also has the potential to eliminate neoplastic cells. The concept of “immune surveillance” was first described more than 50 years ago and refers to the ability of immune cells to detect tumor cells and destroy them. T cells, part of the adaptive immune system, are critical for tumor immune surveillance. Immune surveillance may lead to a period in which cancer cells are kept in check by the immune system, and the tumor neither expands nor regresses. Eventually, tumors develop means to escape immune control. Mechanisms of escape include decreasing the surface expression of antigen-presenting MHC I molecules. This makes the cells effectively invisible to the T cells. Another mechanism is increased expression of programmed cell death ligand 1 (PD-L1) on the cancer cells, which can bind the PD-1 receptor on activated T cells, leading to protection against T cell–mediated killing. Current immunotherapies that target this so-called immune checkpoint have led to long-lasting regression in several cancers.

Chemokines, or chemotactic cytokines, have critical roles in mediating recruitment of immune cells to sites of inflammation and tumors. For example, the C-C chemokine ligand 2 (CCL2) recruits CC chemokine receptor (CCR2)-expressing immune cells to tumors. The chief role of CCR2 in cancer is therefore thought to be the regulation of immune cell infiltration, such as the recruitment of monocytes to tumors after treatment with chemotherapy.

Breast cancer cells also express CCR2, but the effects are largely unknown, in part because they were thought to be minor compared with the roles of CCR2 in host cells. We have now found that Ccr2 in MMTV-PyMT breast cancer cells has a surprising role in inhibiting tumor immune surveillance. This effect is driven by multiple changes in the immune environment, including increased infiltration and activation of cytotoxic T cells and dendritic cells, as well as higher expression of MHC-I and lower expression of PD-L1 on cancer cells. These results establish a novel role for cancer cell CCR2 in suppressing an adaptive immune response, thereby promoting immune escape.

### Neutrophils Promote Breast Cancer Metastasis

**J. Park, R. Wysocki, L. Maiorino, M. Fein, J. Albrengues, V. Kuettner**

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 communications between cancer cells through growth factors and cytokines play an important role in the formation of metastasis.

We compared the microenvironments 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. This led us to identify infiltration of neutrophils as highly up-regulated in metastatic breast cancer.

Neutrophils are the most abundant leukocytes in blood. Neutrophils’ normal function is to kill harmful microorganisms via three modes: (1) phagocytosis, a process whereby bacteria or fungi are engulfed and digested; (2) degranulation of cytotoxic enzymes into the extracellular space; and (3) neutrophil extracellular traps (NETs). These are peculiar scaffolds of DNA with associated cytotoxic enzymes that are released into the extracellular space, where they trap microorganisms. NETs can be formed in tissues and have recently been documented in human pancreatic, liver, and gastric cancers, but whether they play a role in the progression of these cancers remains unclear.

Neutrophils are sensitive to many chemotherapy regimens, resulting in dangerously low neutrophil numbers (neutropenia) during the course of treatment. Neutropenia carries a risk of life-threatening infections, and many cancer patients receiving chemotherapy also receive prophylactic treatment with neutrophil stimulating factors. Understanding the contribution of neutrophils to metastases therefore has pressing clinical implications.

Traditionally, studies on metastasis have relied primarily on measurements made at the end point 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.

To observe how circulating cancer cells and neutrophils interact on arrival in the lungs—a major site of metastatic colonization in breast cancer—we developed confocal intravital lung imaging (CILI), a modification of a lung-imaging approach used with two-photon microscopy. Using CILI, we observed NET-like structures around metastatic 4T1 cancer cells that had reached the lungs of mice. NETs were also found in triple-negative human breast cancer. In vitro, we found that metastatic breast cancer cells can themselves induce metastasis-supporting NETs in the absence of infection. The NETs, in turn, stimulated the invasion and migration of breast cancer cells. These processes were blocked by inhibiting NET formation. Together, our data suggest that induction of NETs by cancer cells is a novel metastasis-promoting tumor–host interaction and a new therapeutic target.

Collagen Architecture in Pancreatic Cancer Progression
M. Shields

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. Type I collagen 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.

Collagen cross-linking in mouse models of mammary carcinoma delays tumor onset and slows tumor progression. Furthermore, collagen architectural structure becomes 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 promotes 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 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
metastatic spread. Intravital imaging in mice showed that pancreatic cancer cells also migrate along collagen fibers, and collagen architecture is altered by cross-linking inhibitors in pancreatic cancer. In addition, we have determined that intracellular signaling pathways, downstream from integrins, are up-regulated after inhibition of LOX in pancreatic cancer cells. This suggests that collagen architecture or the cancer cells' response to collagen is fundamentally different between breast and pancreatic cancer.

PUBLICATIONS


In Press

Pancreatic ductal adenocarcinoma (PDA) is a devastating disease that has responded to the new immunotherapies that have shown promise in other cancers such as melanoma and non-small-cell lung cancer. We are attempting to solve the problem of resistance to immunotherapy by studying a mouse model of PDA. This model replicates the major immunological features of the human disease: resistance to two T-cell checkpoint antagonists, antibodies to CTLA-4 and PD-L1, low immunogenicity because of a low somatic mutational frequency, and a relative paucity of T cells within the tumors. Our approach is informed by the discovery that immunotherapy in the mouse model is enhanced by an inhibitor of a chemokine receptor, CXCR4, that overcomes the block to intratumoral accumulation of T cells. Interestingly, as in human PDA, CXCL12, the ligand for CXCR4, is present on the cancer cells.

How T Cells Are Excluded from the Vicinity of Cancer Cells in PDA

Z. Wang

The exclusion of T cells from the vicinity of cancer cells may be the fundamental means by which epithelial tumors escape immune control and has been reported for human colorectal cancer (CRC), ovarian cancer, and non-small-cell lung cancer, in addition to our observations in human and mouse PDA. The finding by our lab in 2013 that administering AMD3100, a small molecule inhibitor of CXCR4, to PDA-bearing mice induces the rapid accumulation of T cells among cancer cells has prompted a Phase 1 trial of this drug in patients with PDA. We do not, however, know how inhibition of CXCR4 leads to this critical immunological response.

CXCR4 is expressed on all immune cell types and, although it has been reported to be expressed also by some human pancreatic cancers, CXCR4 is not expressed by cancer cells in the mouse model of this disease. Therefore, we are focusing on the role of signaling via CXCR4 on immune cells in blocking the intratumoral accumulation of T cells. First, we have shown that the capacity of AMD3100 to promote immune control of cancer may be extended to transplantable mouse models of lung cancer and CRC, indicating that this CXCR4-dependent means for tumoral immune suppression is not restricted to PDA. Second, we have assessed the transcriptome of the tumor microenvironment (TME) of the mouse C26 model of CRC by RNA sequencing (RNA-Seq), and we discovered that, despite high levels of mRNAs encoding chemokines that should attract all immune cell types, including B cells, T cells, the dendritic cell (DC) subset capable of cross-presenting antigen to CD8+ T cells, natural killer (NK) cells, neutrophils, and monocytes/macrophages, monocytes/macrophages represented the overwhelming majority of cells that were present.

This finding excludes one explanation for the absence of T cells, low intratumoral expression of T-cell-attracting chemokines. It also suggests that the exclusion reaction extends to all immune cell types other than monocyte/macrophages. The biological purpose for this selectivity may be tissue protection because monocytes are required in several examples of tissue regeneration and the excluded cell types would normally have tissue destructive functions. Interestingly, all excluded cell types not only express CXCR4, as do monocytes, but also other members of the CXCR family, such as CXCR2, CXCR3, and CXCR5, which monocytes do not. This observation suggests that exclusion may involve the disabling of immune cells that have been stimulated through CXCR4 and at least one other member of the CXCR family. We are investigating this possibility.

Type I Interferon Signaling and the Immune Suppressive TME of PDA

J. Li

Other investigators have shown that type I interferon (IFN) is required for the immune rejection of immunogenic mouse tumors, and the major pathway
stimulating the production of this cytokine is the stimulator of interferon genes (STING). STING, aka TM173, is an endoplasmic reticulum (ER)-associated protein involved in the sensing of cytosolic DNA, as occurs frequently with viral infections. Among the immunological functions of type I IFN is the stimulation of biosynthesis of CXCL10 by DCs and monocytes, the cytokine that may enable the initial recruitment of CXCR3-expressing T cells, DCs, and NK cells. Activation of these cells in the TME could stimulate the production of IFN-γ, which would amplify the immune reaction by inducing the biosynthesis of CXCL9 and CXCL10, thereby recruiting additional CXCR3-expressing immune cells. Thus, the type I IFN response may be essential for tumor immunity because it may initiate the accumulation of T cells.

The murine C26 CRC tumor and murine PDA differ in that the latter stringently excludes T cells, whereas the former shows infiltration of a modest number of T cells, albeit a number insufficient for control of tumor growth. We have compared the mRNA levels for CXCL9, CXCL10, and IFN-γ in these tumors and found that they were almost 100-fold higher in the C26 tumor. This has led to our considering the possibility that there is a type I IFN response in the C26 tumor but not the PDA. Indeed, analysis of the RNA-Seq data from the C26 tumor revealed elevated expression of type I IFN-regulated genes that were not transcriptionally activated in murine PDA.

We will substantiate these findings in murine and human PDA because they may indicate why PDA is resistant to conventional immunotherapy. Interestingly, because AMD3100 treatment of mice with PDA uncovers sensitivity to anti-PD-L1, we also suspect that this immunotherapy may be efficacious because it leads to the production of type I IFN within the tumor. We will assess this possibility and discover the means by which CXCR4 signaling suppresses the production of type I IFN.

**Immunotherapy Replaces “Exhausted” T Cells by Promoting Intratumoral T-Cell Proliferation**

M. Ludwig

Current explanations for ineffective immune control of tumors propose that the TME causes “exhaustion” of T cells. Although definitions of the cause of T-cell “exhaustion” differ, there is general agreement that repetitive stimulation of the T-cell receptor (TCR) must occur in the absence of clonal expansion, a response that would replace the “exhausted” T cells with progeny that show the functionality of “fresh” T cells.

We have proposed that the exhausted phenotype is a consequence of the exclusion of new immigrants to the TME. These “fresh” effector T cells are continuously generated in the tumor-draining lymph nodes by the interaction of central memory T cells with antigen-bearing DCs arriving via lymphatics draining the tumor. Moreover, if these CXCR3-positive DCs are also excluded from the tumor in a CXCR4-dependent manner, then not only would they be impaired in their ability to pick up cancer-cell-derived antigens for subsequent presentation in the draining lymph nodes, but they would also be impaired in their capacity to drive the proliferation of intratumoral T cells. Thus, the exhausted phenotype reflects the absence of a capacity for intratumoral T cell–DC interaction that would drive the clonal expansion of cancer-specific T cells—not, as suggested by some, a unique capacity of the TME for causing a dysfunctional state of T cells.

To support this proposal, we are conducting experiments in which intratumoral T-cell proliferation in mice bearing PDA is measured in the presence and absence of acute AMD3100 treatment. The contribution of T cells from lymph nodes will be measured by blocking lymph node T-cell egress with the drug FTY720. The requirement for intratumoral DCs will be examined by assessing intratumoral T-cell accumulation after conditionally depleting the subset of DCs capable of cross-presentation of antigen.

**A Model to Study Dormant Pancreatic Cancer Hepatic Metastases**

A. Pommier

Even when patients have PDA surgically removed, the 5-year survival rate is a disappointing 25%, mainly because of the presence of undetected metastases at the time of surgery. The liver is the most frequent site for blood-borne metastases because the pancreas venous drainage is via the hepatic portal vein. We have generated a model of pancreatic cancer hepatic metastasis with several long-term aims, one of which is discussed here.

We considered the possibility that hepatic metastases occurring in the context of an ongoing adaptive
immune response would not grow out as clinically detectable macrometastases because the cancer-specific T cells would kill the disseminated cancer cells before they establish a protective immune suppressive TME. However, we wondered whether some disseminated cancer cells might establish nongrowing, dormant metastases that had escaped immune recognition and would be clinically silent.

A PDA cell line derived from a hepatic metastasis of a KPC mouse was modified by the insertion of three vectors expressing, respectively, luciferase, diphtheria toxin, and herpes simplex thymidine kinase. One group of mice was subcutaneously inoculated with these cancer cells and tumors were allowed to grow for two weeks, during which the mice developed a T-cell response to the PDA. These mice were then treated with diphtheria toxin and ganciclovir, which eliminated the subcutaneous tumors. The modified PDA cells were then injected into the portal circulation of these mice and mice that had not been immunized, and metastatic tumor growth in the liver was evaluated by bioluminescent imaging of the livers. Luciferase-positive macrometastases were observed in all nonimmunized mice by 2 weeks postinjection, but no metastases were detected by this means in livers removed from the preimmunized mice for up to 8 weeks postinjection. Microscopic examination of liver sections from these mice, however, revealed rare, single cells that stained with antiluciferase antibody that resided in the parenchymal tissues without any apparent local inflammation reaction. Thus, the anticancer immune response did prevent the outgrowth of metastatic lesions but did not prevent the establishment of dormant metastases. Intriguingly, the disseminated, single luciferase-positive PDA cells did not express the epithelial marker cytokeratin-19, but did express fibronectin, a marker of mesenchymal cells—strongly suggesting that the dormant cancer cells had undergone at least some aspects of an epithelial–mesenchymal transition.

Having established this model of dormant PDA metastases, we plan to discover the reasons why they have escaped immune killing and the conditions that may lead to outgrowth as macrometastatic lesions.

**PUBLICATIONS**


BET Bromodomain Inhibition Suppresses the Function of Hematopoietic Transcription Factors in Acute Myeloid Leukemia

K. Rivera, D. Pappin [in collaboration with J.-S. Roe, F. Mercan, and C. Vakoc (CSHL)]

Deregulation of chromatin structure and function is a major mechanism that drives the pathogenesis of human cancer, which often occurs through somatic mutation of genes encoding chromatin-modifying enzymes or factors that interact with modified histones. The bromodomain and extraterminal (BET) protein BRD4 is a validated drug target in leukemia, yet its regulatory function in this disease was not understood. In collaboration with the Vakoc laboratory, the Mass Spectrometry Laboratory showed that hematopoietic transcription factors (TFs) perform a critical role in directing BRD4 recruitment to enhancer and promoter regions in acute myeloid leukemia. These effects are mediated by the p300/CBP acetyltransferases, which are recruited by TFs to their occupied sites to maintain lysine acetylation that is critical for BRD4 occupancy. Evidence was found that interactions with both histones and TFs were contributory to the recruitment mechanism of BRD4 in this disease. Crucially, the Pappin laboratory helped identify a histone-like motif on the ERG transcription factor which is specifically acetylated by p300 to mediate a direct interaction with BRD4. Additionally, chemical inhibition of BET proteins suppressed the functional output of hematopoietic TFs in leukemia cells. Because hematopoietic TFs and p300/CBP are essential to maintain the leukemia cell state, shown in this work to be mediated by lysine acetylation, the findings suggest that BET inhibitors exert therapeutic effects in this disease by perturbing lineage-specific transcriptional programs.

Use of a Lys/Arg-N Protease for Mass Spectrometry Applications

J. Wilson, N.S. Turna, D. Pappin

Directed in vacuo peptide fragmentation using mass spectrometry can generate specific ion fragment series that simplify tandem mass spectrometry (MS/MS) collision spectra. This is most readily achieved by controlling where charge is located on the peptide, either enzymatically or by chemical modification. To produce predominantly amino-terminal ions, charge must be placed at or near the amino termini of peptides. This can be elegantly achieved using a proteolytic enzyme with amino-terminal cleavage specificity at basic amino acids, and we have discovered and characterized 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 1–2 hr at 60°C. The enzyme exhibits a temperature optimum of ~60°C and broad pH specificity centered at pH 8.5, with at least 50% activity remaining at pH 5 and pH 11. The protease is most active in low-ionic-strength MS-compatible acetate buffers and requires both calcium and zinc. Specificity for amino-terminal cleavage at arginine and lysine was approximately >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 ~20× shorter digestion times. Eighty percent of the enzyme activity was retained in 0.5% sodium dodecyl sulfate (SDS) and specificity was equal for both lysine and arginine residues. As expected, b-ion series in MS/MS spectra were significantly more intense than y ions, and relative b/y-ion intensities were almost exactly reversed compared to trypsin. In complex mixtures, a shift to lower peptide charge states was also observed. This active metalloprotease is suitable for extremely rapid (1–2 hr) digestion of samples for high-throughput 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. The Pappin MS lab has begun to use the enzyme routinely for characterization of post-translational modifications such as phosphorylation, where the dominant b-ion fragment series have enabled a significantly higher rate of detection.
PTEN Functions by Recruitment to Cytoplasmic Vesicles
S. Bencze, D. Pappin [in collaboration with A. Naguib, H. Cho, W. Zheng, A. Tocilj, E. Elkayam, C. Faehnle, C. Pratt, M. Chen, L. Joshua-Tor, and L. Trotman, CSHL; N. Jaber and W.-Z. Zong, Stony Brook; Michael Marks, Children’s Hospital, Philadelphia]

Historically, PTEN was thought to function at the plasma membrane, where receptor tyrosine kinases are activated. However, the majority of PTEN is located throughout the cytoplasm. Phosphoinositide (PI) lipids constitute a small fraction of total cellular lipids but perform essential roles in signal transduction. Among many other functions, these include propagating signals through a variety of signaling axes.

Using the Pappin laboratory MS capabilities to measure PTEN activity by directly measuring the levels of PI, PIP, PIP2, and PIP3 lipids, the Trotman laboratory was able to completely change this view and understand that PTEN activity was effectively controlled by the singly phosphorylated PI(3)P lipid. They showed that cytoplasmic PTEN was distributed along microtubules tethered to vesicles via phosphatidylinositol 3-phosphate (PI(3)P), the signature lipid of endosomes, and demonstrated that the noncatalytic C2 domain of PTEN specifically bound PI(3)P through the CBR3 loop. Mutations rendered this loop incapable of PI(3)P binding and abrogated PTEN-mediated inhibition of PI3-kinase/AKT signaling. In short, PTEN was inactivated by removing the ability to bind to this specific lipid on the surface of endosomes. Control the level of this PI(3)P lipid, or interfere with the ability of PTEN to bind, and you control PTEN. This loss of function could be rescued by fusion of the loop mutant PTEN to FYVE, the canonical PI(3)P binding domain, demonstrating the functional importance of targeting PTEN to endosomal membranes. Beyond revealing an upstream activation mechanism of PTEN, the data introduced the concept of PI3-kinase signal activation on the plasma membrane that is balanced by PTEN-mediated signal termination on the small, discrete surfaces of internalized endosomal vesicles.

A Quantitative Proteomics-Based Signature of Platinum Sensitivity in Ovarian Cancer Cell Lines
D. Pappin [in collaboration with G. Fan, K. Wrzeszczynski, C. Fu, G. Su, R. Lucito, and N. Tonks, CSHL]

Although DNA encodes the molecular instructions that underlie the control of cell function, it is the proteins that are primarily responsible for implementing those instructions. Therefore quantitative analyses of the proteome would be expected to yield insights into important candidates for the detection and treatment of disease. The Pappin, Lucito, and Tonks laboratories collaborated on an iTRAQ-based proteomic screen of 10 ovarian cancer cell lines and two normal ovarian surface epithelial cell lines. They profiled the abundance of 2659 cellular proteins, of which 1273 were common to all 12 cell lines. Of the 1273, 75 proteins exhibited elevated expression and 164 proteins had diminished expression in the cancerous cells compared with the normal cell lines. The iTRAQ expression profiles allowed us to segregate cell lines based on sensitivity and resistance to carboplatin. Importantly, there was no substantial correlation between protein abundance and RNA expression or epigenetic DNA methylation data. Furthermore, we could not discriminate between sensitivity and resistance to carboplatin on the basis of RNA expression and DNA methylation data alone. The study illustrated the importance of proteomics-based discovery for defining the basis for the carboplatin response in ovarian cancer and highlighted many candidate proteins, particularly those involved in cellular redox regulation, homologous recombination, and DNA damage repair, which otherwise could not have been predicted from whole genome and expression data sources alone.

Differential Connectivity of Splicing Activators and Repressors to the Human Spliceosome

During spliceosome assembly, protein–protein interactions (PPIs) are sequentially formed and disrupted to accommodate the spatial requirements of pre-mRNA substrate recognition and catalysis. Splicing activators and repressors, such as serine/arginine-rich (SR) proteins and hnRNPs, modulate spliceosome assembly and regulate alternative splicing. However, it remains unclear how they differentially interact with the core spliceosome to perform their functions. In collaboration with the Kainer and Zhang laboratories, the Pappin laboratory helped to investigate the protein connectivity of serine/arginine-rich (SR) and heterogeneous ribonucleoprotein particle (hnRNP) proteins to the core spliceosome with a novel computational
approach that used probabilistic network reconstruction based on the integration of interactome and gene-expression data. The model was validated using immunoprecipitation and mass spectrometry of the prototypical splicing factors SRSF1 and hnRNPA1. Network analysis revealed that a factor’s properties as an activator or repressor could be predicted from its overall connectivity to the rest of the spliceosome. In addition, we discovered and experimentally validated interactions between the oncoprotein SRSF1 and members of the antitumor drug target SF3 complex. The findings suggested that activators promote the formation of interactions between spliceosomal subcomplexes, whereas repressors mostly operate through protein–RNA interactions.

PUBLICATIONS


ROLE OF CROSS TALK BETWEEN INTERLEUKIN-6 AND TRANSFORMING GROWTH FACTOR β1 IN CHEMORESISTANCE

R. Sordella  A. Gray  M. Miller  N. Pisterzi  P. Safaric Tepes  N. Shirole
N. Korimerla  D. Pal  N. Sachan  S. Senturk  Z. Yao

The focus of the Sordella laboratory is how to identify novel therapies for the treatment of lung cancer. Lung cancer is one of the leading causes of death worldwide, with mortality rates exceeding those of breast, prostate, and colon cancers combined. This is, in part, because of a lack of effective treatment and the highly aggressive nature of non–small cell lung carcinoma (NSCLC), which accounts for the large majority of all cases of lung cancer.

Nongenetic Determinants of Drug Resistance: What Doesn’t Kill Me Makes Me Stronger

Resistance to therapy is one of the main impediments to the clinical efficacy of cancer treatments. In recent years, we have been using a multifaceted approach to try to identify novel drug-resistant mechanisms with the ultimate goal of translating our findings in the clinic and improving cancer outcomes. As an initial paradigm, we have used lung cancer cells driven by oncogenic epidermal growth-factor receptor (EGFR) mutations. This choice was based on our previous work demonstrating that although the EGFR tyrosine kinase inhibitor (TKI) erlotinib was highly effective in the treatment of NSCLC harboring oncogenic EGFR mutations, the majority of patients eventually would develop resistance and succumb to the disease.

TGF-β Inhibition of MCP1P1 Is Required for Cancer Drug Resistance and Metastatic Colonization

In NSCLC-derived cell lines and erlotinib-naïve tumors driven by oncogenic EGFR mutations, we have uncovered the existence of a subpopulation of cells that are intrinsically resistant to erlotinib and display features suggestive of epithelial-to-mesenchymal transition (EMT). We showed that these cells were generated by a stochastic/epigenetic mechanism and the activation of TGF-β-mediated signaling was sufficient to induce EMT and drug resistance. Because IL-6 and TGF-β are prominently produced during inflammatory response, by using a mouse model system, we showed that induction of inflammation not only stimulated IL-6 secretion but was also sufficient to decrease the tumor response to erlotinib. These findings are exciting because they indicate that the development of resistance to EGFR TKI might occur not only as a consequence of the acquisition of de novo mutations, but also that nongenetic factors could impair the response of patients to EGFR TKI. This concept is important because it argues that treatments based only on EGFR inhibition may not be sufficient for the effective treatment of lung-cancer patients harboring mutant EGFR. Although these findings were initially viewed with skepticism, many new studies have now provided additional evidence in support of our studies. In particular, clinical data have now confirmed that the mechanism we described can explain 15% of cases of acquired resistance in patients undergoing treatment with EGFR TKI.

Although we showed the importance of the TGF-β IL-6-axis in mediating acquired resistance, the detailed mechanistic aspects were still unclear. In a recent study, we found that TGF-β inhibition of ZC3H12A was essential in regulating IL-6 expression. ZC3H12A is a bifunctional endo-ribonuclease and deubiquitinase protein that controls the stability and expression of multiple cytokines. We showed that TGF-β direct inhibition of ZC3H12A transcription regulated the expression of IL-6 via a bimodal mechanism encompassing increased mRNA stability and mRNA transcription. In the latter case, this occurred by releasing the activation of nuclear factor kappa-B (NF-κB), a sequence-specific transcription factor that is best known as a major regulator of inflammatory and innate immune responses. From a biological standpoint, regulation of IL-6 by ZC3H12A was dispensable for TGF-β-induced EMT, but it was instead required for
TGF-β-induced drug resistance and metastatic colonization of cancer cells. These findings open novel opportunities for the treatment of NSCLC. Importantly, a recent clinical trial by Gilead has opened to test the efficacy of a dual NF-κB/JAK2 inhibitor in combination with erlotinib for the treatment of mutant EGFR-positive tumors. At the same time, the increased activation of NF-κB also suggests that the inhibition of MCPIP1 by TGF-β could be important for the expression of other cytokines and, more generally, in orchestrating the dialog of cancer cells with the surrounding tumor microenvironment.

As described, we have now uncovered novel molecular mechanisms by which cancer cells become either intrinsically resistant or acquire resistance to cancer treatment. Excitingly, these findings have opened new therapeutic opportunities that are currently being tested in the clinic.

**TGF-β Endows CD44+/CD24− Cells with Increased Genetic Instability and Adaptability by Weakening DNA Double-Strand Break Repair Mechanisms**

Given the importance of the drug-resistant/mesenchymal cells, we conducted a functional genomic short hairpin RNA (shRNA)-based screen to better understand the vulnerabilities of these cells. Surprisingly, we observed that the mesenchymal/EGFR TKI-resistant cells were particularly sensitive to the inactivation of genes participating in DNA repair. We found this was a result of intrinsic defects in their DNA double-strand break (DSB) repair capability. In particular, in these cells, the constitutive activation of the TGF-β axis was required and sufficient for reducing the expression of genes that play a critical role in coordinating DNA damage repair such as BLM, BRCA2, FANCF, NBN, PMS1, RAD50, RDM1, WRN, ATM, and ATR. This resulted in an increased accumulation of DNA copy number alterations, genetic diversity of cancer cell populations, and an improved adaptability to drug treatment. Because these cells are naturally occurring and can be generated by a stochastic/epigenetic program, our findings suggest that the interconversion between different cell states can promote intratumor genetic heterogeneity, spur the tumor’s evolution, and hence increase the tumor’s fitness. This is important because it argues that the transition into a drug-tolerant/mesenchymal state could provide a mechanism that allows a small subpopulation of tumor cells to withstand an initial destructive attack of drug to enable their survival, until they will accumulate more permanent resistance mechanisms. We are now revisiting the data from their original EGFR TKI clinical trial to try to understand whether increased genetic instability could be a signature that could be used to predict response to therapy and time to relapse. Accumulation of mutations has been shown to correlate with response to immune-checkpoint-based therapies. We are also trying to determine whether the lung tumors with high content of drug-tolerant/mesenchymal cells are the ones sensitive to immune-checkpoint inhibitors.

**p53-psi, the Protector Gene’s Evil Twin Linked to Spread of Cancer**

*p53-psi Is a Transcriptionally Inactive p53 Isoform Able to Reprogram Cells toward a Metastatic-Like State*

TP53 is one of the most intensively studied genes. The p53 tumor suppressor plays a critical role in genome surveillance, suppression of oncogenic transformation, and regulation of many fundamental aspects of both reversible and irreversible cellular stress responses. TP53 mutations, through loss of p53 function and putative gain-of-function activities, disable these effects and are associated with aggressive cancers and poor patient prognosis. Although we were investigating a possible contribution of p53 in EGFR TKI resistance, we unexpectedly identified a completely novel, naturally occurring p53 isoform generated by an alternative-splicing event. This isoform was not expressed in normal tissue, but it was highly abundant in tissue on chronic injury, and it was enriched in highly metastatic NSCLC. Consistent with these observations, we found that, although lacking transcriptional activity and canonical tumor suppressor functions, p53-psi is able to reprogram cells toward the acquisition of metastatic features via a Cyclphilin D (CpD) interaction in the mitochondria matrix. Our results thus identify a new mode of p53 regulation and suggest a physiological role of this p53 isoform in tissue injury repair. We are now following up these studies and investigating how p53-psi alternative splicing is regulated, how it changes the activity of CpD, why the CpD and p53-psi interaction increases the production of mitochondria reactive oxygen species (ROS), and how the latter can induce the drastic phenotypic changes we observed on p53-psi expression.
Truncating Alleles in TP53 Produce Separation of Function Mutants That Actively Promote Tumorigenesis

TP53 truncating mutations are common in human tumors and are thought to give rise to p53-null alleles. Hence, we asked whether some of these mutations could mimic p53-psi activities. We showed that among all TP53 truncating mutations, only the TP53 exon-6 occur at higher than expected frequencies; they are expressed and produce proteins that functionally and molecularly resemble p53-psi. Accordingly, we found that TP53 exon-6 truncating mutants lack canonical p53 tumor suppressor activities but instead promote cancer cell proliferation, survival, and metastasis even in the absence of a wild-type p53 allele. Similar to p53-psi, these mutants can also localize to mitochondria—where they promote tumor phenotypes by binding and activating the mitochondria inner pore permeability regulator, CpD. This suggests a possible physiological origin of certain p53 mutations and indicates that mutations resulting in the generation of truncated p53-psi-like proteins—contrary to the current dogma—do more than create a 53-null state. This is an important breakthrough because it indicated that p53-psi-like isoforms, rather than halting tumorigenesis, promote it. The discovery of selective dependencies of cancer cells harboring p53 exon-6 truncating mutations to CpD activity is particularly exciting as it provides a rationale and an opportunity for the design of novel targeted therapeutics. The high prevalence of p53-psi-like mutations in tumors also argues that this class of p53 mutations represents a strong precision medicine candidate target comparable in frequency to well-recognized EGFR, ROS, and ALK mutations in NSCLC. Notably, ~4% of unselected cancer patients harbor these particular mutations, although in some tumors such as head and neck, pancreatic, ovarian, lung, and colon cancers, p53-psi-like mutations are present in ~10% of patients.

Development of Tools for the Functional Characterization of Genes In Vivo

A Rapid and Tunable Method to Temporally Control Cas9 Expression Enables the Characterization of Essential Genes and the Interrogation of Functional Gene Interactions In Vitro and In Vivo

Over time, it became apparent that the possibility of acutely inactivating genes in cells and organisms was essential to our studies. In the context of cancer research, we were particularly interested in the identification and characterization of essential genes, as they could provide potential therapeutic targets. Yet, the fact that upon their inactivation the cells die renders them the most difficult class of genes to study. Although many different approaches have been developed and used in the past, none could simultaneously (1) provide potent, robust, and temporally controlled gene inactivation, (2) be applied to a broad spectrum of cell types and tissues, (3) facilitate high-throughput manipulation, and (4) be traceable. By exploiting a strategy developed by the Wandless lab, we showed that by conjugating a genetically encoded FKBP12-derived destabilizing domain to Cas9 (DD-Cas9) we could achieve a highly specific, conditional regulation of Cas9 expression and temporal control of gene editing. We designed the system in such a way that it could be easily adapted to coexpress, from the same promoter, DD-Cas9 with any other gene of interest without comodulation of the latter. In particular, when coexpressed with inducible Cre-ERT2, our system enables parallel, independent manipulation of alleles targeted by Cas9 and traditional recombinase with single-cell specificity. We anticipate this platform will be used for the systematic characterization and identification of essential genes and the interrogation of genes’ functional interactions.

Summary

In summary, our work has produced important breakthroughs in our understanding of drug resistance mechanisms and intratumor heterogeneity, which resulted in novel therapeutic opportunities. At the same time, unexpectedly, our studies have also uncovered novel biology surrounding one of the most studied genes, the tumor suppressor p53, that has changed the old dogma that all p53 truncations generate null alleles. As described, these findings have also generated new and even more exciting questions. In addressing them, we will be able to significantly contribute to scientific understanding of molecular mechanisms driving tumorigenesis and to transform current cancer treatments.

Publication

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 catalyze 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 functions of these enzymes are frequently disrupted in human diseases. The ability to modulate such signal transduction pathways selectively with drugs holds enormous therapeutic potential. In the area of tyrosine phosphorylation—dependent signal transduction, drug discovery efforts to date have emphasized the protein tyrosine kinases (PTKs); however, considering the reversibility of protein tyrosine phosphorylation, there is the potential to manipulate signal transduction pathways at the level of both PTKs and protein tyrosine phosphatases (PTPs). Although the PTPs have been garnering attention as potential therapeutic targets, they remain largely an untapped resource. The long-term objectives of the work of our lab are to characterize the structure, modes of regulation, and physiological function of members of the PTP family of enzymes. Through the application of basic research to understand the mechanism of PTP action, our lab is trying to devise creative new approaches to exploit these enzymes as targets for therapeutic intervention in major human diseases, including diabetes, obesity, and cancer.

During the last year, Dipikaa Akshinthala left the lab to work with Senthil Muthuswamy in Toronto, Mathangi Ramesh completed her PhD and has moved on to become an Associate Consultant at IMS Consulting Group with the goal of working in health care, and Ming Yang completed her PhD and has taken up a postdoctoral position in the Schneider lab at New York University (NYU). Christy Felice, Kevin Nicholas, and Om Shrestha have joined the lab as technicians.

### Small Molecule Inhibitors of PTP1B as Therapeutic Agents

At the core of this effort is the study of PTP1B, the prototypic PTP, 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 not only to address diabetes and obesity but also mammary tumorigenesis and malignancy. PTP1B plays a positive role in promoting signaling events downstream from the oncoprotein tyrosine kinase HER2; consequently, small molecule inhibitors of PTP1B may represent a novel therapeutic strategy for treatment of tumorigenesis and malignancy associated with elevated HER2. Previously, our lab showed that Trodusquemine/MSI-1436 is a novel allosteric inhibitor of PTP1B, which attenuates HER2-dependent tumorigenesis and abrogates metastasis in the NDL2 mouse model of breast cancer. Now, the U.S. Food and Drug Administration (FDA) has approved testing of MSI-1436 in a Phase 1 clinical trial in metastatic breast cancer patients, in collaboration with the Northwell Health Monter Cancer Center.

In the past year, our lab has been examining the effect of MSI-1436 on tyrosine phosphorylation in HER2-positive breast cancer cells, such as BT474, to define its mechanism of action. Within the field, PTPs are still frequently viewed as broad specificity, housekeeping enzymes; however, contrary to such a view,
the effects of MSI-1436 on tyrosine phosphorylation are subtle. Currently, we are comparing the effects of MSI-1436, and other PTP1B inhibitors, with the effects of suppressing the enzyme by RNA interference (RNAi) and CRISPR-Cas, with the goal of identifying critical substrates of PTP1B.

To date, much of the focus on therapeutic development has been on inhibitors of protein kinases such as Herceptin for HER2. Although we represent breakthroughs in cancer therapy, not all patients respond, and those who do soon develop resistance to the drug. Our lab has been examining whether inhibition of PTP1B with MSI-1436 will be an effective approach to overcoming resistance. Over the past year, we have generated cells with acquired resistance to Herceptin and have shown that MSI-1436 inhibits the growth of these cells in culture. In addition, we know that combinations of MSI-1436 and lapatinib are more effective in inhibiting tumor growth in xenograft studies with HER2-positive BT474 breast cancer cells than either compound alone. Currently, we are extending this analysis of tyrosine phosphorylation also to include the impact of MSI-1436 on Herceptin-resistant states and in cells cultured to become resistant to MSI-1436.

Although MSI-1436 shows efficacy in an injectable format, like many PTP1B inhibitors identified to date it also has limited oral bioavailability. During the last year, our lab characterized a derivative of MSI-1436 for which we have shown oral bioavailability; however, there are some unique features of its mechanism of action that distinguish it from the parent molecule. Analysis of its mechanism of action is continuing. Furthermore, our lab has identified a structurally distinct allosteric inhibitor that appears to act through the same mechanism as MSI-1436. This is currently being validated as an inhibitor of PTP1B, together with other novel chemical entities that display similar properties, to identify additional new drug candidates.

A New Role for PTP1B as a Target for Therapeutic Intervention in Rett Syndrome

In studies of PTP1B function, Nava Krishnan in our lab identified this phosphatase as a therapeutic target for Rett syndrome (RTT). This is an X-linked neurological disorder presenting with autistic features, which are caused primarily by mutations in a transcriptional regulator, methyl–CpG-binding protein 2 (MECP2). Current treatment options for RTT are limited and focus on alleviating some of the neurological symptoms. We showed that the PTPN1 gene, which encodes PTP1B, is a target of MECP2, and that disruption of MECP2 function was associated with increased levels of PTP1B in RTT models. Pharmacological inhibition of PTP1B ameliorated the effects of MECP2 disruption in mouse models of RTT, including improved survival in young male (Mecp2−/−) mice and improved behavior in female (Mecp2+/−) heterozygous mice. Furthermore we showed that PTP1B was a negative regulator of tyrosine phosphorylation of the tyrosine kinase TRKB, the receptor for brain-derived neurotrophic factor (BDNF). Consequently, the elevated levels of PTP1B that are generated by disruption of MECP2 function in RTT would be expected to represent a barrier to BDNF signaling. In fact, inhibition of PTP1B led to increased tyrosine phosphorylation of TRKB in the brain, which would augment BDNF signaling. Taken together, this work presents PTP1B as a mechanism-based therapeutic target for the treatment of RTT, validating a novel strategy for treating the disease by modifying signal transduction pathways with small-molecule drugs.

Redox Regulation of PTP Function

PTP1B is a major regulator of the signaling pathways initiated by insulin, which controls glucose uptake and metabolism, and leptin, which controls appetite. Gene targeting studies showed 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 obesity effects in rodents, as well as human subjects. Another major project in the lab is to validate a novel approach to the development of inhibitors of PTP1B as drug candidates for the treatment of diabetes and obesity, which represent, perhaps, the greatest health-care challenge facing the nation. Major programs in industry have focused on developing small-molecule inhibitors...
of PTP1B to promote insulin and leptin signaling in resistant states. Nevertheless, these efforts have been frustrated by technical challenges arising from the chemical properties of the PTP active site. In particular, although it is possible to generate potent, selective, and reversible active site-directed inhibitors, the tendency for such molecules to be highly charged, such as pTyr-substrate mimetics, presents problems with respect to their oral bioavailability and limits their drug development potential. As a result, the industry views PTP1B, and other PTPs, as challenging. Consequently, innovative strategies are required to generate inhibitors of this highly validated target that may be readily exploited for drug development.

In this project, the goal is to harness a physiological mechanism for redox regulation of PTP1B function that reflects a new tier of control of tyrosine phosphorylation-dependent signaling. Previously, our lab observed that the activity of PTP1B is attenuated by reversible oxidation of an essential cysteiny! residue at the active site of the enzyme. The architecture of the PTP active site is such that this essential cysteiny! residue displays unique properties that favor its role as a nucleophile in catalysis but also render it prone to oxidation. Insulin stimulation of mammalian cells leads to enhanced production of intracellular H₂O₂, which causes reversible oxidation of PTP1B and inhibition of its enzymatic activity, which, in turn, concomitantly promotes the signaling response to insulin. We 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. Previously, we used phage display to identify conformation-sensor antibodies that recognize the reversibly oxidized form of PTP1B (PTP1B-OX) selectively and stabilize this inactive state, inhibiting its reactivation by reducing agent and thereby inhibiting phosphatase activity. We showed that expression of these antibodies in cells enhanced insulin-induced signal transduction. These 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.

Now, the mechanistic basis for the interaction of these antibodies with PTP1B-OX is being defined. A cluster of predominantly basic residues close to the

![Figure 1. Model to illustrate MECP2-mediated regulation of BDNF-TRKB signaling. MECP2 suppresses expression of PTP1B, which augments BDNF-induced signaling through the TRKB PTK. Functional loss of MECP2 in Rett syndrome (RTT) results in an increase in levels of PTP1B, which attenuates BDNF-TRKB signaling. (Taken from Krishnan et al. 2015.)](attachment:image.png)
active site were shown to be critical for mediating binding between the conformation-sensor antibody scFv45 and PTP1B-OX.

Although the antibodies themselves are unlikely to be of use as therapeutics, our 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 and bind to this site on PTP1B-OX. In a proof-of-concept study, we screened the LOPAC library of compounds (Sigma-Aldrich) for small-molecules that would stabilize the oxidized form of PTP1B and inhibit its reduction and reactivation. This pilot screen revealed two such hits, sanguinarine and levamisole. These compounds were repurchased and further characterized. We targeted the oxidized form of PTP1B selectively over the reduced form of the enzyme. Furthermore, although the two inhibitors targeted PTP1B-OX, we did not affect its closest paralog, TCPTP. Ten analogs of sanguinarine were purchased, from which chelerythrine was identified as having higher affinity for PTP1B-OX and being without effect on TCPTP. Interestingly, one compound from this series of molecules was unable to inhibit the reduction and reactivation of PTP1B, suggesting the beginning of a structure-activity relationship. These compounds will be tested further in cell-based assays and animal models of insulin and leptin signaling.

Characterization of Receptor PTPδ as a Potential Therapeutic Target in Tumors Characterized by Loss of Missing-in-Metastasis

This year, our lab published a paper illustrating that both SRC and PTPδ may be considered therapeutic targets for metastatic tumors associated with loss of missing-in-metastasis (MIM), which is a scaffold protein that is down-regulated in multiple metastatic cancer cell lines compared with nonmetastatic counterparts. To pursue this further, we have been trying to generate antibodies to PTPδ. Initial attempts have been frustrated by the fact that the intracellular portion of PTPδ is highly conserved across species. Now, we are focusing on generating antibodies to the extracellular segment of this receptor PTP, which may also serve as probes by which to modify the function of the enzyme.

Efforts to Define How Loss of Expression of Specific PTPs that Regulate Cell Migration and Invasion In Vitro Contribute to Tumorigenesis In Vivo

Previous work from our lab identified PTPN23 as an important regulator of mammary epithelial cell migration and invasion. In addition, the PTPN23 gene is located on a chromosome region, 3p21, that is spontaneously lost in 8%–10% of breast cancers, and low expression of PTPN23 coincides with poor survival. In an orthotopic transplantation model, we observed that when PTPN23-deficient Comma 1Dβ cells were transplanted in the cleared mammary fat pad, PTPN23-induced severe primary tumor development in 52 weeks (18 out of 24) was suppressed, and a few cases (two out of 24) of lung metastasis occurred. Immunohistochemistry analysis detected extensive phosphorylation of Tyr 142 in β-catenin in tumor samples but not in mammary glands repopulated with control cells or in normal mammary glands. We have now shown that the primary effects of PTPN23 in this context are exerted through FYN, a SRC family kinase. Under normal conditions, PTPN23 dephosphorylates the auto-phosphorylation site in FYN, thereby suppressing its activity. Following suppression of PTPN23, FYN activity is elevated, leading to enhanced phosphorylation of Tyr 142 in β-catenin. Recent studies have revealed that treatment of mice bearing PTPN23-deficient cells with AZD0530, a small-molecule inhibitor of SRC family kinases, reversed the effect of PTPN23 suppression on tumor growth. These data suggest that FYN may be a good therapeutic target for tumors defined by loss of heterozygosity (LOH) of PTPN23.

Use of CRISPR-Cas9-Based Gene Targeting to Define PTP Function in Acute Myeloid Leukemia

During this year, a unique opportunity presented itself to collaborate with Chris Vakoc and the “Functional Genomics” Shared Resource of the Cancer Center. This followed logically from a previous systematic analysis of PTP function using RNAi conducted in our lab. Working in collaboration with the Shared
Resource, our lab generated a pooled single-guide RNA (sgRNA) library that targets 101 mouse PTPs and allows them to examine the function of members of the PTP gene family systematically in various models. Aberrant tyrosyl phosphorylation–dependent signaling is associated with cell proliferation, antiapoptosis, and drug resistance in leukemia progression. In this project, we decided to identify and characterize acute myeloid leukemia (AML)-dependent PTPs. Using the CRISPR-Cas9 gene-editing tool, we performed a negative screen by infecting an AML cell line, RN2, with a pooled sgRNA library that targets 101 mouse PTPs. By comparing the sgRNA abundance at the beginning and end of culture by MiSeq sequencing, our screen revealed a small set of PTPs that are required for RN2 cell proliferation and survival, including PTPN23, DUSP12, and PTPMT1. Current studies are focused on validating these PTPs for their role in AML.

**PUBLICATIONS**


Resistance to therapy of metastatic prostate cancer (CRPC) is responsible for the annual deaths of some 250,000 U.S. men. Although there is considerable progress in the development of improved antihormone therapy for treatment of metastatic disease, this standard of care approach will invariably fail at some point.

Our focus is to understand the mechanisms driving human prostate cancer in its most lethal form: metastatic disease. We have studied the human genetics behind the transition from indolent to lethal metastatic prostate cancer and combined it with viral transgene delivery into prostate. With this approach, we have succeeded in generating a unique, fast, and faithful mouse model for metastatic prostate cancer. We have termed this system RapidCaP, as it allows us to generate any genetically mutant mouse prostate cancer with a much accelerated time frame compared with breeding-based approaches. Now, we use RapidCaP for analysis and therapy of metastatic disease.

At the same time, we aim to better define PTEN/PI3-kinase signaling and derive new types of markers that indicate high metastatic potential of disease.

**Endocytic Vesicles Are Discrete Platforms for PTEN Signaling**

A. Naguib, H. Cho, T. Herzka, W. Zheng, C. Pratt, M. Chen [in collaboration with N. Jaber and W. Zong, Stony Brook University; M. Marks, Children's Hospital of Philadelphia; G. Bencze, D Pappin, A. Tocilj, E. Elkayam, C. Faehnle, and L. Joshua-Tor, CSHL]

PTEN is the major negative regulator of PI3-kinase signaling, with cell-specific functions that go beyond tumor suppression. We aim to better define PTEN/PI3-kinase signaling at its most fundamental level. This has led us to probe PTEN function in the cytoplasm, where the vast majority of PTEN resides. We use live imaging, superresolution microscopy, and fluorescence photobleaching to map and capture PTEN/PI3-kinase signaling in motion.

PTEN is proposed to function at the plasma membrane, where receptor tyrosine kinases are activated. However, the majority of PTEN is located throughout the cytoplasm. Using the above approaches, we could show that cytoplasmic PTEN is distributed along microtubules, tethered to vesicles via phosphatidylinositol 3-phosphate (PI(3)P), the signature lipid of endosomes. We showed that the noncatalytic C2 domain of PTEN specifically binds PI(3)P through the CBR3 loop. Mutations render this loop incapable of PI(3)P binding and abrogate PTEN-mediated inhibition of PI3-kinase/AKT signaling. This loss of function is rescued by fusion of the loop mutant PTEN to FYVE, the canonical PI(3)P-binding domain, demonstrating the functional importance of targeting PTEN to endosomal membranes.

Beyond revealing an upstream activation mechanism of PTEN, our data introduce the concept of PI3-kinase signal activation on the vast plasma membrane that is contrasted by PTEN-mediated signal termination on the small, discrete surfaces of internalized vesicles. Conceptually, there are several interesting aspects to an alternative of PI3-kinase signal termination on vesicle membranes, as opposed to stochastic termination on the plasma membrane. First, a vesicle with a 100-nm diameter has a surface area that is five orders of magnitude smaller than the surface of a typical plasma membrane. It can be envisioned that this smaller surface and constitutive PTEN-vesicle binding makes PTEN-substrate interaction far more likely, processive, and controllable. Second, our findings may introduce a major missing link in the spatiotemporal control of PI3-kinase signal termination. APPL1-positive endosomes have been shown to serve as a platform for receptor tyrosine kinase (RTK)-mediated mitogen-associated protein kinase (MAPK)/AKT signaling, and a recent study identified PI(3)P...
deposition as an essential switch for removal of APPL1 from vesicles and termination of vesicle signaling. Our findings could, therefore, link the termination of AKT signaling on APPL1-positive endosomes to recruitment of PTEN by PI(3)P deposition on endosomes. Our results suggest an unexpected link between PTEN function, its localization, and the endocytic compartment. Intriguingly, this association was first hinted at 17 years ago, when the PTEN/MMAC1 gene was originally identified and two research teams independently noted that PTEN has significant sequence similarity to auxilin. Auxilin is essential for endocytosis: It is recruited to clathrin-coated vesicles precisely after they pinch off from the plasma membrane to mediate the disassembly of the clathrin coat. This is essential for the incoming vesicle membranes to be recognized and sorted. The event is tightly timed to ensure that clathrin lattice uncoating only occurs after vesicles have separated from the plasma membrane.

There are two remarkable consistencies between auxilin’s structure and function and our findings on PTEN-vesicle interaction. First, it has been shown that the PTEN-like domain of auxilin is essential for recruitment of the protein to the vesicle membrane. The crystal structure of this domain of auxilin has been determined. It is nearly identical to the structure of PTEN itself. This finding is consistent with a conserved vesicle-binding function between the two homologous domains. Second, several analyses showed that the C2 domain in auxilin mediates the essential vesicle interactions, whereas the catalytic domain is dispensable based on mutation analysis. Most notably, it was found that auxilin’s equivalent of the PTEN CBR3 loop (termed “loop 3” in auxilin) is critical for binding to PI(4)P—and (and the other monophosphorylated phosphatidylinositol phosphates (PIPs): PI(3)P and PI(5)P).

Collectively, these data are consistent with the intriguing possibility that the PTEN C2 domain may have evolved to recognize intracellular vesicle membranes based on their monophosphorylated PIP concentration. This concentration can be efficiently enriched and presented on the limited surfaces of vesicles after internalization from the cell periphery. This process can prevent massive dilution of the signaling lipid over the plasma membrane to facilitate protein recruitment.

**Cell Communication in Metastasis**

D. Nowak, K. Watrud, A. Ambrico, C. Stahlhut [in collaboration with J. Wilkinson, University of Michigan; B. Robinson, Weill Cornell Medical College; M. Castillo-Martin and C. Cordon-Cardo, Mt. Sinai School of Medicine]

Resistance to therapy of metastatic prostate cancer (CRPC) is responsible for the annual deaths of some 250,000 U.S. men. Although there is considerable progress in development of improved drugs for treatment of metastatic disease, it remains a huge challenge to predict which patients will best respond to a given approach. The RapidCaP system is highly versatile, as genetic alterations are introduced by infection of the mouse prostate through transgene-carrying virus. In a first approach, we used RapidCaP to model loss of the Pten and p53 genes, a signature that has been found in 50% of human castration-resistant metastatic prostate cancer. In contrast to the classic approaches of studying (human) tumors “in” a mouse, our approach allows us to study prostate cancer “of” a mouse. RapidCaP makes use of existing genetic engineering tools with conditional tumor suppressor alleles. We trigger disease by viral Cre delivery and monitor progression of disease by integration of the luciferase transgene and noninvasive visualization using bioluminescence imaging. It exceeds current genetically engineered mouse (GEM) prostate models by producing focal disease that retains intact histopathology of surrounding stroma and normal tissue. In contrast, traditional conditional knockout of the entire prostatic epithelium, and targeting the same genes, results in lethal primary disease burden within months. This artificial scenario is without human counterpart and has so far hampered analysis of progression phenotypes.

The RapidCaP system allows for discovery and validation of new metastasis driver genes and progression principles. We discovered the switch from Akt to Myc activation in prostate metastasis to lung. The disease showed absence of Akt activation but high Myc levels in cytoplasm and nuclei of all metastatic nodules. We also noted that the mock castrated Pten/Trp53-deficient RapidCaP prostate showed weak or absent and exclusively nuclear Myc, similar to normal prostate (not shown). Our data showed that c-Myc was spontaneously activated to drive metastasis and CRPC, in contrast to disease initiation, in which we observed strong Akt activation.
We next investigated the mechanism behind these results. Our findings identified a critical role for cell–cell communication through the secreted IL6 cytokine, which activates STAT3 signaling to promote MYC as a driver of Pten/Trp53–mutant metastasis. The MYC gene is frequently amplified in prostate cancer, and functional validation established it as a driver of prostate cancer and its metastasis, as seen using the RapidCaP system. Human genome analysis revealed that MYC alterations usually stem from broad amplifications on chromosome 8q and correlate with metastatic prostate cancer (57% in metastasis vs. 15% in primary prostate cancer). Our work suggests that cell–cell communication is intimately linked to genetic changes of PTEN and TP53. It is known that chemokines and interleukins change their expression patterns during prostate cancer progression. Now, our in vitro and in vivo data specifically link the gene loss of PTEN and TP53 to IL6 secretion and metastasis to soft tissues.

PUBLICATIONS


We investigate the fundamental biology of pancreatic ductal adenocarcinoma (PDA) and use this information to formulate new clinical approaches for this highly lethal malignancy. As an enabling technology, we recently developed organoid tissue models of human and mouse PDA, to complement PDA genetically engineered mouse models (GEMMs). The PDA organoids have provided new biological insights and identified new therapeutic opportunities. Additionally, we are initiating a large organoid biobanking effort to better define the genetic makeup and pharmacological vulnerabilities in human PDA. Finally, GEMMs and organoid models of PDA are currently being evaluated as the platforms to launch our new Preclinical Experimental Therapeutics-X Facility (PETx), with an immediate task of determining whether organoids can facilitate early stage human trials.

Protein Translation as a Redox Dependency in Pancreatic Cancer
This work was done in collaboration with D. Pappin and M. Hammel (CSHL); N. Sonenberg (McGill).

Although reactive oxygen species (ROS) potently affect cancer cell proliferation and influence therapeutic responsiveness, the critical biochemical pathways that are deterministic toward cell proliferation as opposed to cell death are still unclear. Using mouse models and organoids, we found that oncogenic Kras induces the Nrf2/Nfe2l2 transcription factor to stimulate pancreatic cancer proliferation and to maintain the viability of malignant cells. Nrf2 is the master regulator of the antioxidant response and works by directing the transcription of genes that promote a reduction in intracellular ROS levels. By establishing a sensitive redox proteomics method, we identified translational regulatory proteins to be specifically oxidized when Nrf2 was deleted from pancreatic cancer cells. Both cap-dependent and -independent mRNA translation is impaired in Nrf2-deficient pancreatic cancer cells, because of both the direct oxidation of the protein translation machinery and the impairment of mitogenic signaling, and both antioxidants and exogenous mitogens can mitigate this defect. Pro-oxidants partially phenocopy the redox properties of Nrf2 by lowering the glutathione pools, and they potently synergize with inhibitors of PI3K/AKT to promote pancreatic cancer cell death in vitro and in vivo. Thus, mRNA translation is particularly impaired by elevated reactive oxygen species in pancreatic cancer, presenting a synthetic lethal strategy. We are now optimizing the therapeutic approaches that target protein translation in PDA patients, for consideration of imminent evaluation in patients.

Stromal Architecture and Heterogeneity in Pancreatic Cancer
This work was done in collaboration with R. Hynes (MIT).

A dense desmoplastic stroma is characteristic of PDA tumors. This stroma contains an abundance of extracellular matrix proteins, and scattered non-neoplastic cells include immune cells, pancreatic stellate cells, and endothelial cells. To investigate the nature of the stroma, we have investigated the biochemical composition of matricellular proteins in collaboration with Richard Hynes at MIT. Using a sensitive proteomic method, we have characterized the composition of the matrix in human and mouse normal pancreatic tissues, pancreatitis, and early and advanced invasive pancreatic tumors. Our cross-species comparison has identified previously known matricellular proteins such as collagens and fibronectin and has also revealed novel proteins. We have applied mRNA in situ hybridization techniques to determine whether the neoplastic or stromal cells express these novel transcripts and have
discovered genes expressed exclusively in either compartment. Current work is aimed at determining the functional significance of these genes, using genetic approaches in mice and organoids that are then transplanted orthotopically. In addition to our work with the stroma, we have developed a coculture system containing organoids and pancreatic stellate cells (PSCs). In coculture, the PSCs become activated and secrete a dense extracellular matrix. Furthermore, coculture supports the proliferation of each cell type and the passing of each in limiting conditions. PSCs in direct contact with epithelial organoid cells are stimulated to express myofibroblastic genes, whereas those distant from the epithelial cells assume a secretory fate (Fig. 1).

These observations have been confirmed in tissue samples of human and mouse PDA and may represent the heterogeneous behavior of PSCs that sculpts the creation of the tumor microenvironment in this disease. The function of the different subtypes of PSCs will be dissected using organoids and in vivo models, with a goal of determining whether the distinct subtypes can be manipulated for therapeutic benefit.

**Therapeutic Development for Pancreatic Cancer**

This work was done in collaboration with C. Thompson (Memorial Sloan Kettering Cancer Center).

With the opening of PETx, we are poised to develop new strategies that identify active therapies for pancreatic and other types of cancer. Over the past year we had a productive collaboration with Craig Thompson, where we helped determine the tumor-promoting effects of rapamycin in PDA. We are currently exploring the development of oxidants and other targeted strategies using our organoids and mouse models.

**PUBLICATION**

Research in my laboratory is focused on the role of Ras and Rho GTPase family members in signal transduction. Ras and Rho family members play key roles in cellular activities controlling cell growth, differentiation, and morphogenesis. Alterations in Ras and Rho functions have been causally linked to both cancer and brain/mental disorders, including intellectual disability, schizophrenia, epilepsy, and mood disorders. 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, their regulators, and effectors in models of cancer and neurodevelopmental/neurological disorders. Below are highlighted selected key projects.

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

Among the various cortical interneuron subtypes, chandelier cells (ChCs), in particular, are believed to have powerful control over the output of pyramidal neurons (PyNs), because of their unique morphology and the types of connections they make. ChCs possess a very distinctive axonal arbor with multiple arrays of short vertical sets of cartridges, each harboring a string of synaptic boutons. This unique architecture enables a single ChC to couple to a large population of PyNs. Furthermore, ChCs selectively innervate the axon initial segment (AIS) of PyNs, the most excitable part of a neuron in which the action potential is initiated. Together, these characteristics make ChCs ideally suited to exert powerful control over a large group of PyNs. Significantly, changes in ChC cartridges/boutons and/or function have been reported in disease states such as schizophrenia and epilepsy. To date, however, details on ChC cartridge/bouton development remain scarce, and hardly anything is known about the molecular mechanisms that govern the morphological differentiation of ChCs. The main obstacles have been a lack of unique biochemical ChC markers and, in particular, versatile methods to target and manipulate gene expression in these cells. Based on 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 (IUE) directed toward the vMGE, and we found that this is indeed the case. Having the methodology in hand that allows us to modify gene expression in ChCs, we next tackled the identification of molecular mechanisms that govern ChC cartridge/bouton development. In ongoing studies examining the expression of selected members of the DOCK180 family, an atypical class of Rac and/or Cdc42 GTPase guanine nucleotide exchange factors (GEFs), in GABAergic interneurons, we intriguingly observed the presence of the DOCK7 family member in ChCs of adolescent/adult mouse brains. Significantly, mutations in DOCK7 have been reported in individuals with epileptic encephalopathy. To examine DOCK7's role in ChCs, we combined the above-described IUE approach with RNA interference (RNAi) to knock down endogenous DOCK7 protein levels. We found that knockdown of DOCK7 caused a disorganization of ChC cartridges and a decrease in the density and size of ChC boutons, indicating an essential role for DOCK7 in ChC cartridge/bouton development. 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.

In more recent studies, we also embarked on defining the molecular components that govern ChC innervation selectively at the AIS of cortical PyNs. Although interfering with DOCK7-ErbB4 signaling leads to a decrease in the density and size of ChC boutons, we observed that ChC cartridges of DOCK7 and ErbB4 knockdown cells still made contact with the AISs of neighboring PyNs. This raised the question as to how specific contact formation between ChC cartridges and the AIS of PyNs is achieved. To assess whether it is signals from the AIS that attract ChC cartridges, we first tested whether disruption of the AIS structure prevents ChCs from innervating PyNs. To this end, we performed a double IUE approach to separately knock down AnkG in cortical PyNs and label ChCs with a fluorescent marker. We found that none of the PyNs had ChC cartridges innervated on their axons, supporting our hypothesis that signaling molecules on the AIS of PyNs are important for ChC innervation. To gain insights into the identity of these proteins, we are currently taking a candidate gene approach, with a focus on axonal proteins with adhesion functions, including Eph receptors, members of L1 protein family, TAG1, and Caspr2. Elucidation of the mechanisms that regulate ChC selective innervation at the AIS will not only provide novel insight into the processes underlying ChC synapse formation, but also help to elucidate the mechanisms underlying the association between alterations in ChC innervation and neuropsychiatric disorders such as schizophrenia and epilepsy.

Multifunctional Role of the X-Linked Intellectual Disability (XLID) Protein Oligophrenin-1 in Cognition and Behavior

Oligophrenin-1 (OPHN1), which encodes a Rho-GTPase activating protein, was the first identified Rho-linked intellectual disability (ID) gene. It was initially identified by the analysis of a balanced translocation t(X;12) observed in a female patient with mild ID. Subsequent studies revealed the presence of OPHN1 mutations in families with a syndromic form of ID, with affected individuals commonly showing mild to severe ID and behavioral problems. 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. To gain insight into how mutations in OPHN1 could contribute to the cognitive deficits in individuals with OPHN1 mutations, we initially focused on defining the role for OPHN1 in hippocampal plasticity associated with learning and memory. By temporally and spatially manipulating OPHN1 gene expression at the hippocampal CA1 synapse, we uncovered that during early development OPHN1 plays a key role in activity-dependent maturation and plasticity of hippocampal excitatory synapses. In addition, we obtained evidence that OPHN1 also plays a critical role in mediating metabotropic glutamate receptor–mediated long-term depression (mGluR-LTD) in CA1 hippocampal neurons. Indeed, we found that mGluR-LTD induction elicits rapid dendritic OPHN1 synthesis, which is essential for mGluR-LTD, as acute blockade of OPHN1 synthesis impedes LTD. Moreover, we uncovered that this role of OPHN1 is separable from its effects on basal synaptic strength. Thus, our data unveiled a multifunctional role for OPHN1 at CA1 synapses. Independent of its role in activity-driven glutamatergic synapse development, regulated OPHN1 synthesis plays a role in mGluR-dependent LTD. Thus, our findings provided insight into the cellular basis by which mutations in OPHN1 could contribute to the cognitive deficits in OPHN1 patients.

Besides the above-described role for OPHN1 in hippocampal plasticity and spatial learning, we more recently discovered that it also plays a critical role in moderating stress-induced depressive-like behaviors in the learned helplessness (LH) model of depression. In this model, animals are exposed to an unpredictable and uncontrollable stressor and, subsequently, evaluated for their coping capabilities (i.e., resilient vs. helpless/depression-like behavior) in a testing session that consists of an aversive but escapable stressor. We found that Ophn1-deficient mice subjected to the LH procedure show a marked increase in helpless behavior, when compared with their control littermates. Importantly, Ophn1-deficient mice did not display altered motor activity, and were capable of learning and performing the avoidance task. On further examination of the neuronal cell types involved, we found that Ophn1 deficiency in parvalbumin (PV), but not somatostatin (SOM), interneurons promotes
TGF-β/Smad Signaling through DOCK4 Facilitates Lung Adenocarcinoma Metastasis

The cytokine transforming growth factor (TGF)-β plays an important, albeit complex, role in epithelial tumorigenesis. During the early stages of tumorigenesis, TGF-β typically functions as a tumor suppressor; at later stages, however, it can act as a potent promoter of multiple steps of the metastatic process, which comprise local motility/invasion, entry of cancer cells into the blood stream (intravasation), exit from the blood vessels (extravasation), and colonization of distant organs. The relevance of TGF-β signaling for disease progression has been particularly recognized in tumors in which cancer cells retain the core TGF-β signaling components, as is frequently the case in breast and lung cancers. However, a major remaining challenge is the identification of TGF-β target genes that drive the different steps of metastasis, especially because TGF-β modulates gene expression in a highly cell- and context-specific manner. Although some progress to this end has been made in the context of breast cancer metastasis, the genes and mechanisms that mediate the prometastatic effects of TGF-β in lung adenocarcinoma (ADC) remain largely unknown.

To explore molecular mechanisms that could mediate the prometastatic effects of TGF-β in lung ADC, we took a candidate gene approach and started off by scrutinizing members of the DOCK180-related protein superfamily, which, as mentioned above, emerged as a distinct class of Rac and/or Cdc42 effectors. We found that in lung ADC, cells’ expression of DOCK4, but not other DOCK180-family members, is rapidly and robustly induced by TGF-β in a Smad-dependent manner. Moreover, we uncovered that DOCK4 is a direct TGF-β/Smad target gene and, importantly, that high DOCK4 expression correlates with activated TGF-β signaling and poor prognosis in human lung ADC. These findings prompted us to assess the role of DOCK4 in mediating the prometastatic effects of TGF-β in lung ADC in vivo in a mouse model. We found that DOCK4 induction is essential for TGF-β-driven lung ADC metastasis. Specifically, we observed that blockade of TGF-β-induced DOCK4 attenuates the ability of lung ADC cells to extravasate into distant organ sites, resulting in a marked reduction in metastatic burden in mice. At a cellular level, we obtained evidence that TGF-β-induced DOCK4 signal transduction facilitates extravasation by stimulating lung ADC cell protrusive activity, motility, and invasion, without affecting epithelial-to-mesenchymal transition (EMT) and, intriguingly, that it does so by driving Rac1 activation. So far, Rac1 has only been linked to TGF-β via a noncanonical pathway. Thus, our findings identify the atypical Rac1 activator DOCK4 as a novel, key component of the TGF-β/Smad pathway that promotes lung ADC cell extravasation and metastasis.

More recently, we expanded this line of research toward identifying genes that mediate lung ADC organ-specific metastases, with a particular focus on those that mediate colonization of specific organs by lung ADC cells. To this end, we already established multiple organ metastasis model systems in mice by intracardially injecting lung ADC cell populations derived from primary tumors from a KrasG12D/p53−/− mouse model. In collaboration with Dr. Kenneth Chang, we are currently performing RNAi screens in the above experimental model systems to identify mediators of metastatic colonization. Identification of such mediators would not only lead to novel insights into the mechanisms underlying organ-specific colonization by lung ADC cells, but also to the identification of targets for prevention of metastasis growth.

PUBLICATIONS


Malignant glioma is the most common and lethal type of brain tumor. In its most aggressive form, glioblastoma (GBM) has a median survival of 12–15 months after initial diagnosis. Our laboratory studies the genetic and epigenetic programs governing pathogenesis of malignant glioma. And our long-term goal is to define the molecular and developmental programs behind the complex biology of glioma initiation and progression, so as to enable the medical community to develop rational therapeutic strategies targeting this dreadful disease. During the past year, we have continued to focus our research around two major areas: (1) to develop genetically engineered animal model systems to recapitulate the genetic and pathological aspects of various human glioma subtypes and to utilize these glioma animal models as tools to dissect in vivo tumor propagation and their response to experimental therapeutic treatments; and (2) to identify the genetic and epigenetic pathways governing normal and neoplastic neural stem and glioma cell fate determination. Below are some of the highlights of our progress.

Understanding EGFR-Targeted Resistance Mechanism in Glioma Treatment

Malignant glioma pathogenesis is driven by the accumulation of genetic and genomic alterations. The most common lesion is gene amplification, mutation, and overexpression of epidermal growth factor receptor (EGFR). About 50% of EGFR-amplified malignant gliomas also concurrently express EGFRviii, an in-frame deletion EGFR mutant that exhibits ligand-independent constitutive activity. The frequent EGFR gene amplification/mutation and its prominent role as a signaling trigger of many downstream cascades make EGFR a compelling candidate for targeted therapy. The fact that EGFR tyrosine kinase inhibitors (TKIs) have emerged as effective therapies for lung cancer patients carrying active EGFR mutations provides further rationale to replicate the success in aberrant EGFR signaling-driven malignant gliomas. But despite their robust therapeutic benefits to lung cancer patients, the same EGFR TKIs have shown limited efficacies in glioma clinical trials, even to patients with EGFR amplification/mutations. The molecular mechanism(s) behind this poor response remain poorly understood. There is an urgent medical need for uncovering the resistance mechanisms in order to identify efficient therapeutic targets and target combinations and to translate such discoveries into meaningful clinical applications.

In an attempt to elucidate the mechanisms underlying anti-EGFR resistance in GBM treatment, we have collaborated with scientists at Columbia University, Weill Cornell Medical College, and MD Anderson Cancer Center to develop a glioma mouse model driven by inducible expression of the EGFRviii mutant. Three features of this system are notable: (1) this animal model, which comprises the same genetic components as in the correspondent human GBMs, recreates histologically similar malignant gliomas; (2) this mouse glioma system successfully models the heterogeneous EGFR expression pattern observed in human malignant gliomas; and (3) the inclusion of genetic regulatory element enables in vivo manipulation of EGFRviii expression, which allows us to experimentally exploit glioma maintenance function of EGFR signaling. Aided by this powerful model system, we have revealed that oncogenic EGFR signaling-driven gliomas respond to EGFR inhibition in a three-phase response pattern—an initial robust regression is followed by a period of dormancy before the eventual relapse in which tumors progress independently of EGFR signaling. We further demonstrated that EGFR expression plays a glioma maintenance role independent of its kinase activity upon EGFR TKI treatment. We therefore concluded that EGFR TKI resistance in gliomas is mediated through the combination of EGFR-dependent and EGFR-independent mechanisms, and future therapies targeting both of these activities will be required for achieving better treatment outcome.

We recently have begun to further uncover the molecular mechanisms underlying the EGFR-dependent and -independent TKI resistance mechanisms. In col-
laboration with the Tonks lab, we found that EGFR can be phosphorylated independent of its kinase activity by non–receptor tyrosine kinases (nTKIs) and function to activate EGFR downstream signaling. These findings suggest that nTKIs may contribute EGFR TKI resistance by compensating for inhibited EGFR kinase activity. In parallel, we demonstrated that induction of cell fate transition may function to abrogate EGFR signaling dependency and confer on tumor cells anti-EGFR resistance. Together, our studies shed important light on the molecular mechanisms of EGFR-targeted therapeutic resistance in treatment of malignant gliomas. We are currently striving to identify therapeutic strategies to overcome the resistance.

Characterizing ATRX Functions during Neural Development and Glioma Pathogenesis

α-thalassemia/mental retardation syndrome X-linked (ATRX) is a chromatin remodeling factor of Snf2 family, and its germline mutation causes an X-linked developmental disorder characterized by mental retardation, developmental delay, and α-thalassemia. Functionally, ATRX forms a histone chaperone complex with its binding partner death domain associated protein (DAXX) to facilitate replication-independent deposition of histone variant H3.3 at heterochromatic regions. Loss-of-function ATRX mutations are frequently found in many human malignancies, including adult and pediatric glioma, neuroblastoma, pancreatic neuroendocrine tumor, and sarcomas. In human adult glioma, it is noted that ATRX mutations are specifically enriched in low-grade gliomas and derived high-grade gliomas. Loss of ATRX in human tumor and cell lines also tightly correlates with abnormal telomere maintenance and their usage of recombination-based alternative lengthening of telomeres (ALT) for chromosomal end preservation. But how ATRX exerts its tumor suppressor and telomere maintenance functions remains unclear.

In a previous collaboration with the Lowe and Vakoc groups, we initiated a project to probe chromatin remodelers controlling neural stem cell (NSC) and/or glioma cell self-renewal and differentiation. By conducting a screen of a shRNA library targeting epigenetic regulators, we identified ATRX whose depletion promotes mouse glioma cell growth specifically under differentiation induction condition. Using CRISPR-Cas9 or short-hairpin RNA (shRNA)-mediated approaches, we further revealed that depletion of ATRX expression in mouse NSCs strongly promotes in vivo glioma formation, demonstrating that ATRX is a bona fide glioma tumor suppressor. Mechanistically, our analysis of NSC lineage differentiation and RNA-seq data indicates that ATRX plays a crucial role controlling neural development, consistent with our observation that Atrx is highly expressed in neuronal cells compared with other lineages of cells in adult mouse brains. The fact that ATRX expression level increases along the neuronal differentiation path, with the strongest expression confined to postmitotic neuron cells, further supports our idea that ATRX is required for proper induction and/or maintenance of neuronal differentiation state during central nervous system (CNS) development.

ATRX/DAXX Loss Induces Activation of Alternative Lengthening of Telomeres

Telomere maintenance is essential for cell immortalization and tumorigenesis. Although the majority of human malignancies rely on reactivation of telomerase, 10%–15% of them employ recombination-directed ALT to protect chromosomal ends. Despite accumulated evidence that links ALT with loss of histone variant H3.3 chaperone components ATRX and DAXX, the biological significance of this association remains unclear.

In collaboration the Dr. Lieberman’s group at Wistar Institute, we recently demonstrated that depletion of ATRX or DAXX in human NSCs and glioma cells disrupts normal telomeric DNA replication and induces ALT. We found that the ALT activation is contingent on dysfunctional telomeric H3.3 loading. Reconstituted wild-type DAXX, but not mutants defective of either H3.3 or ATRX interaction, abolishes ALT in DAXX-deleted cells, linking ALT activation to defective assembly of telomeric nucleosomes displaced outside of S phase. Along with ALT, we found that ATRX or DAXX deletion also elicits persistent telomeric DNA damage response (DDR) that negatively impacts cell growth. Mitigation of telomeric DDR by ectopic hTERT overexpression restores proliferative capacity of ATRX- or DAXX-deleted cells.
without abolishing ALT induction. Our studies establish ALT as a telomeric repair mechanism activated in response to disrupted telomere replication and suggest telomeric dysfunction-induced DDR as a checkpoint control limiting progression of human ATRX-mutant low-grade gliomas.

**PUBLICATION**

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 the environment across different sensory modalities, combining olfactory, visual, and 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.

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.

Josh Huang and colleagues study the assembly and function of neural circuits in the neocortex of the mouse. The neocortex consists of a constellation of functional areas that form a representational map of the external (sensory, social) and internal (visceral, emotional) world. These areas are strategically interconnected into elaborate information processing networks that guide behavior. The group’s overarching hypothesis is that, at the cellular level, cortical processing streams and output channels are mediated by a large set of distinct glutamatergic pyramidal neuron types, and functional neural ensembles are regulated by a diverse set of GABAergic interneuron types. Understanding cortical circuit organization requires comprehensive knowledge of these basic cellular components. The Huang lab uses state-of-the-art genetic approaches to systematically target cell types and facilitate the application of a full set of modern techniques for exploring cortical circuits. Among GABAergic interneurons, the chandelier cell is one of the most distinctive cell types that controls pyramidal neuron firing at the axon initial segment. Huang and colleagues are studying the developmental specification, activity-dependent circuit integration, and functional connectivity of chandelier cells, an entry point toward understanding a local circuit module. Regarding pyramidal neurons, they are systematically characterizing the developmental origin, axon projection pattern, and input connectivity of multiple classes of pyramidal neuron types, focusing on the forelimb motor cortex. They combine a range of approaches that include genetic and viral engineering, genetic fate mapping, gene expression profiling, cellular imaging, electrophysiology, and behavior analysis. Recently, they began to integrate their studies in the context of the motor cortex control of forelimb movements.

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 to analogous behaviors in human subjects. Currently, the team’s research encompasses study of (1) the neural basis of decision confidence, (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 Koukalov 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 are not known are 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 Koukalov and his team.

Understanding the link between neural circuits and behavior has been the focus of research in Bo Li’s lab. Li and colleagues are particularly interested in studying the synaptic and circuit mechanisms underlying reward processing, attention, and learning and memory, as well as synaptic and circuit dysfunctions responsible for maladaptive behaviors that are related to major mental disorders. They integrate in vitro and in vivo electrophysiology, imaging, molecular, genetic, optogenetic, and chemogenetic techniques to probe and manipulate the function of specific neural circuits—with a focus on the fear and reward circuits—in the rodent brain, and to determine how these circuits participate in adaptive or maladaptive behavioral responses in various tasks.

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, approximately 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.

Jessica Tollkuhn’s lab seeks to understand how transient events during brain development exert lasting effects on gene expression, circuit function, and, ultimately, behavior. They study how sex-specific neural circuits in rodents are established and modulated by the gonadal hormones estrogen and testosterone. The cognate receptors for these hormones are nuclear receptor transcription factors, which orchestrate modification of the local chromatin environment and thus
exert long-term effects on gene expression. However, the genes regulated by these receptors, as well as the specific mechanisms they utilize, remain poorly understood in the brain. This is in part because the extraordinary cellular heterogeneity of the brain complicates analysis of the small subpopulations of neurons that mediate sex-specific behaviors.

Having recently identified sex differences in both gene expression and chromatin in brain regions known to regulate sex-specific behaviors, the Tollkuhn lab is now working to understand how hormones generate these molecular sex differences during development, through the use of biochemical, genomic, and behavioral analyses. They have developed a method that permits genome-wide analysis of histone modifications or DNA methylation in genetically defined populations of neurons. They hypothesize that these epigenetic data, combined with gene expression profiling, define the molecular signature of the critical period for sexual differentiation of the brain. Their goal is to provide a mechanistic link between the transcriptional effects of hormone signaling during development and the consequent social behaviors displayed in adulthood.

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, researchers 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 SENSORIMOTOR TRANSFORMATIONS IN OLFACTION

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The focus of our research group is on understanding how neuronal circuits encode and interpret input from the environment across different contexts, senses, and brain states to generate 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 use the rodent olfactory system as a model and monitor neuronal inputs, outputs, and feedback loops across different layers of the circuit.

We want to understand (1) how inputs get processed at different synapses of the underlying neuronal circuits; (2) what roles top–down feedback signals play in decoding specific stimulus features as a function of prior experience; and (3) how neuronal representations change across brain states and contexts to support cognitive processes such as selective attention. The broad scope of this effort is observing how perceptions arise and relate to action during specific behaviors.

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 OB

In the sensory bulb, neurons expressing the same type of olfactory receptor converge in tight focus, forming approximately 2000 clusters of synapses called glomeruli. The layout of glomeruli on the bulb is highly reproducible across individuals with a precision of 1 part in 1000. However, nearby glomeruli are as diverse in their responses to odors as distant ones, lacking an apparent chemotopic arrangement (Soucy et al. Nat Neurosci 12: 210 [2009]). From each glomerulus, a few dozen mitral cells (principal output neurons of the OB) carry the output further to the olfactory cortex and several other brain areas. Such “sister” mitral cells typically have only one primary dendrite that projects to a single glomerulus, but can sample inputs on their primary and secondary dendrites from functionally diverse glomeruli via several types of interneurons. Using optogenetic manipulations and digital micro-mirror device (DMD)-based patterned photostimulation, we found that sister cells carry different information to the cortex: average activity representing shared glomerular input, and phase-specific information that refines odor representations and is substantially independent across sister cells (Dhawale et al. Nat Neurosci 13: 1404 [2010]).

Activity in the bulb is a rich mix of excitation and inhibition, via both direct inputs and feedback connections. Many different classes of interneurons interact with the sensory afferents and/or the output neurons via both short- and long-range connections. Their connectivity patterns and roles in olfactory processing remain, however, largely unknown. We use a Cre/loxP approach to express reporters (i.e., synapticPHluorin, GCaMP6) and light-gated switches of neuronal activity (ChR2, Halorhodopsin, Arch) in different bulbar neuronal types. We monitor the inputs (glomerular activity patterns) and the outputs (mitral cell firing) in response to numerous odorants across a wide range of concentrations (three orders of magnitude), as we systematically perturb the activity of the input nodes (glomeruli) and different interneuron types. Patterned photo-stimulation allows us to bypass odor stimulation and gain precise spatiotemporal control over the inputs by directly activating glomerular patterns of choice. We record bulb outputs via multitetrode recordings and patch clamp, or 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: “dopaminergic/GABAergic cells” (DAT+) which broadcast long-range signals in the glomerular layer and “granule cells” (GCs), which establish reciprocal synapses with mitral/tufted cells and receive rich cortical feedback input.

**Long-Range Interactions between DAT+ Cells and External Tufted Cells Implement Gain Control and Gate the Output of the Glomerular Layer of the Bulb**

Odors elicit distributed activation of input nodes (glomeruli) on the OB. This necessitates long-range interactions between coactive glomeruli. Long-range projecting dopaminergic/GABAergic cells (DAT+) provide the earliest opportunity for such cross talk. DAT+ 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. Computational models have suggested that these 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 find that DAT+ cells convey concentration-dependent odor signals by broadcasting focal glomerular inputs throughout the OB. Using optogenetic and pharmacogenetic approaches, we find that DAT+ cells influence distant M/T cells by modulating local excitatory interneurons (ET cells). Synaptic action of DAT+ cells enables long-range suppression of ET cells. In addition, we find electrical coupling between DAT+ and ET cells, which may facilitate local synchronization of ET cell responses. Our results indicate that ET cells are essential gatekeepers of glomerular output and prime determinants of M/T responsiveness. Further, using a cell-specific ablation approach, we show that long-range DAT+-to-ET action implements gain control and contrast enhancement via M/T cell odor response decorrelation, by allowing multiple coactive inputs to alter the output of any given glomerulus (Fig. 1) (Banerjee et al. 2015).

**Characterization of GC 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 bulb. GCs receive glutamatergic inputs both from MT cells, as well as from feedback axons originating in the olfactory cortex. GCs are the most numerous cells in the bulb (~90%), outnumbering the MT 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, owing to technical difficulties in electrophysiological recordings from these small-sized neurons.

To begin characterizing the odor response properties of GCs, we 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 (Fig. 2). GCs showed robust spontaneous activity and were sparsely activated on odor presentation, displaying a diverse range of enhanced and suppressed, ON, OFF, as well as ON–OFF responses. Enhanced responses were more common than suppressed ones (65% vs. 35%). Further, a significant fraction (~25%) of GCs showed 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 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 cortico-bulbar 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
Figure 1. DAT+ cells implement gain control and decorrelate mitral cell odor responses. (A) TH+ immunohistochemistry in the olfactory bulb (OB) of a DAT-Cre x Thyl-GCaMP3.0 mouse injected with AAV2.9-FLEX-DTR-GFP virus on the dorsal aspect of the OB followed by diphtheria toxin (DT) intraperitoneal injection. Confocal images of the TH+ signal in two representative fields of view (FOVs) on the ventral (left) and dorsal (right) aspect of a given slice. Average counts of TH+ cells from all dorsal FOVs and from all ventral FOVs in DTR-injected mice, normalized by the number of visible glomeruli (100% for control and 8.3% for +DT, n = 1259 cells, eight FOVs, two mice). Residual signals mostly consisted of neuropil. (B) Baseline-subtracted, normalized GCaMP3.0 ensemble mitral cell responses to increasing concentrations of allyl tiglate. (Top) Day 0 before injection of DT (control, n = 33 cells). (Bottom) Different FOVs in the same OB on Day 7 after DT injection (+DT, n = 44 cells). Each row represents an individual mitral cell (ROI) in the same FOV. Gray color indicates (dF/F). Dotted lines indicate odor presentation (4 s). (C) (i) Distribution of onset latencies of mitral cell odor responses for control (1.55s ± 1.51s, n = 3660 cell-odor pairs) and +DT condition (1.2s ± 1.39s, n = 3000 cell-odor pairs). Numbers denote mean and standard deviation. Both the mean (two-sample t-test, p < 10⁻¹⁸) and the variance (F-test, p < 10⁻⁵) are significantly smaller under the +DT condition. (ii) Odor-evoked response (dF/F) of four example mitral cells from B, as a function of odor concentration. (iii) Mean odor-evoked response (dF/F) of all mitral cells from B as a function of odor concentration for Allyl tiglate on Day 0 (black, n = 33 cells) and Day 7 after +DT administration (gray, n = 44 cells). (D) Mean odor-evoked response (dF/F) of all mitral cells pooled across experiments as a function of odor concentration for Allyl tiglate and Ethyl valerate. Concentration response curves are shown for control (black, n = 168 cells, seven FOVs, seven mice) and +DT conditions (light gray, n = 150 cells, seven FOVs, three mice). (E) Cumulative distribution of odor response strength of all mitral cells pooled across experiments, to five odors as a function of concentration under the control condition (n = 168 × 5 cell-odor pairs for each concentration) and after +DT injection (n = 150 × 5 cell-odor pairs for each concentration). (F) Cumulative distribution of slopes of the ensemble mitral cell concentration response curves separately fitted for each FOV and odor, under the control and +DT conditions (n = 35, seven FOVs × five odors). (G) Population sparseness of mitral cell odor representations for each concentration under the control (black, n = 168 cells, seven FOVs) and +DT conditions (gray n = 150 cells, seven FOVs). Each bar denotes the mean population sparseness for five odors at each concentration. (H) (i) Matrix of correlation coefficients between the neural response spectra (length of vector = number of recorded neurons) for each odor pair at 1:100 dilution under the control and +DT conditions. (ii) Distribution of pairwise correlation coefficients between all stimulus pairs (n = 190 pairs, five odors at four concentrations) under the control and +DT conditions.
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, 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 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 produce feature-specific perturbations in a glomerular pattern of choice. We aim to understand what features of an odor map are used by a behaving mouse in naturalistic conditions to identify odors and their attributes, such as concentration, temporal fluctuations, or spatial location. Further, 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 (see Fig. 5H). Animals are reinforced with water reward for correct choices and a mild air puff as punishment on licking the wrong port (see Fig. 5H). 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 nontarget stimuli (not A) as mice learn and perform the task. These activity patterns are further used as templates to design photo-stimulation 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.”

**OB Coding of Puffs, Mixtures, and Sniffs**

The olfactory system receives intermittent and fluctuating inputs arising from dispersion of odor plumes and active exploration by the animal. The highly nonlinear olfactory transduction machinery and complex excitatory-inhibitory circuitry are believed to generate a nonlinear population trajectory of neuronal activity that distinguishes odorants. In contrast, we recently showed (Gupta et al. 2015) that individual M/T cells linearly sum inputs across odors and time. By decoupling odor sampling from respiration, we showed that M/T cell responses to arbitrary odor waveforms are well described by an odor-specific impulse response convolved with the odorant’s temporal profile (Fig. 3). This linear convolution applied to respiratory airflow predicts the classical inhalation-locked firing of OB neurons and several other reported response properties of M/T cells. This raises interest in further understanding how this linear transform is achieved given the complexity of local microcircuits in the bulb and feedback from other brain areas. These results suggest that the OB linearly processes fluctuating odor inputs, thereby simplifying downstream decoding of stimulus identity and temporal dynamics.

**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

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**Figure 3.** Schematic of the linear model. (A) The M/T cell response is calculated by convolving the odor kernel with the odor waveform (measured via PID). Negative firing rates are rectified after addition of the preodor baseline. Vertical bars mark odor ON periods. (B) Estimated odor kernels of three example M/T cells. Odors used are indicated at the left of each kernel. Odors were delivered at 1% saturation. (C) Comparison of model predictions and observed responses of the M/T cells in (B) to various time-varying odor stimuli. Vertical gray bars mark odor ON periods. Black lines show the observed mean firing rate across 12 trials. Gray bands indicate SEM. Red lines show the nonrectified prediction from the model using the estimated kernels shown in (B). M/T, mitral/tufted; SEM, scanning electron microscopy.
stimulus, rodents readily identify odors essential for their survival against varying odor-rich backgrounds, and faithfully track a fluctuating odor stream to its source. Several models have attempted to explain invariant perception in different sensory modalities. Some of these models are based on the conventional view of sensory processing that relies solely on feed-forward information flow, whereas others argue that feed-forward mechanisms are insufficient and suggest the involvement of feedback between different neuronal layers. Anatomical studies show massive feedback projections from higher brain areas to the sensory periphery that often outnumber direct sensory inputs. The interplay of feed-forward and feedback signals has been proposed to be fundamental for learning and memory recall.

The OB receives rich glutamatergic projections from the piriform cortex. However, the dynamics and importance of these feedback signals remain unknown. We use multiphoton calcium imaging to monitor cortical feedback in the OB of awake mice and further probe its impact on bulb output. We found that responses of feedback boutons are sparse, odor specific, and often outlasted stimuli by several seconds. Odor presentation both enhanced and suppressed the activity of boutons. However, any given bouton responded with stereotypic polarity across multiple odors, preferring either enhancement or suppression. Feedback representations were locally diverse and differed in dynamics across bulb layers. Pharmacological inactivation of piriform cortex increased odor responsiveness and pairwise similarity of mitral cells, but had little impact on tufted cells (Fig. 4). We propose that cortical feedback differentially impacts the two output channels of the bulb by specifically decorrelating mitral cell responses to enable odor separation (Otazu et al. 2015).

We further aim 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.

**Monitoring Cholinergic Input in the OB 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 its spatiotemporal statistics have been, however, 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.

To circumvent these issues, we are taking advantage of genetically engineered mice (Chat-Cre) and viral strategies to target expression of calcium indicators (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. 5C,D), 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 vs. mitral cell layer); and (2) fiber-optic imaging in freely moving mice (Fig. 5E–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 bulb is linked to the timing and nature
of olfactory stimuli across different behavioral states. We investigate how cholinergic surges in the OB during odor detection, discrimination, and multimodal reversal learning tasks (switching reward contingencies between olfactory and auditory stimuli) modulate the output of the OB and relate to behavioral performance (Fig. 5).

Implementing DMD-Based and Digital Holography Methods to Investigate in a Closed-Loop Fashion the Spatiotemporal Integration Rules in the OB 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 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 (OB 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.

Figure 5. (A) Sustained attention (i) and selective attention (ii) tasks. (B) 1-mo-old cranial window over MOB; cholinergic axons expressing GCaMP5 imaged at 100-μm depth (150×150-μm field of view). (C) Recorded traces of spontaneous activity of cholinergic axons from three different ROIs (4 min, mean dF/F = 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 optic fiber-imaging setup. (G) Two proofs of principle odor-evoked responses acquired with optical fibers from cortical feedback axons expressing GCaMP3 (left, 6-sec odor; black bar, mean dF/F = 3%, four repeats each) recorded in the main OB. MOB, mouse olfactory bulb; OB, olfactory bulb.

Figure 6. Combined imaging and photostimulation setup. (Left) Experimental configuration, combining scanning two-photon imaging (red), DMD photostimulation, and holographic photostimulation. (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 photostimulate deeper (<500 μm) into the brain with cellular resolution. Calcium activity is monitored in an independent optical plane by two-photon imaging and electrodes in downstream brain regions. BE, beam expander; SM, scan mirrors; O, objective; PMT, photomultiplier; SLR, camera lens; DMD, digital micromirror device; SLM, spatial light modulator; CCD, charged-coupled device.
Mapping Odor Space onto Neuronal Representations in the Olfactory System

Unlike many other sensory systems, low-dimensional metrics for characterizing stimuli have remained elusive for olfaction, and it is unclear what features of chemical stimuli are represented by neurons. We would like to relate neural activity in the early olfactory system of mice to the physicochemical properties of odorants. We imaged odor-evoked responses in identified tufted and mitral cells in awake mice using multiphoton microscopy. Similarity of odors computed through a comprehensive set of 1664 physicochemical features was a poor predictor of similarity of the corresponding neuronal representation by mitral or tufted cells. Dimension reduction revealed that fewer dimensions (approximately 14) in the combined neural-chemical space than in neural space alone (approximately 25) were required to explain 90% of variance in neural responses across the population, suggesting that factors other than the physicochemical properties we considered are required to fully explain the neural responses (Fig. 7). Responsive mitral and tufted cells were spatially dispersed, and cells within...
a local region were functionally heterogeneous with only limited and variable dependence of M/T cell position on odorant characteristics. Our data indicate that novel descriptors are needed to link chemical space to neuronal representations and that odor information leaves the bulb in a mosaic pattern with substantial local diversity (collaboration with the Koulakov lab).

Other Collaborative Projects with CSHL Groups

We are collaborating with other CSHL scientists on the following projects: DLP-based patterned stimulation to functionally map the motor cortex (Z.J. Huang); sequencing the OB—bridging the gap between glomerular odor responses and odor receptor sequences by identifying the molecular identity of glomeruli (A. Koulakov, J. Lee, A. Zador); a fiber-optic-based approach to monitor neuronal activity in punishment and reward neuronal circuits during behavior, with a focus on monitoring and manipulating activity in the lateral division of central amygdala and the insula cortex (B. Li); 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).

PUBLICATIONS


In Press

INVESTIGATING NEURAL CIRCUITS FOR SENSORY INTEGRATION AND DECISION-MAKING

A. Churchland  B. Cascone  M. Kaufman  F. Najafi  O. Odoemene
L. Chartarifsky  A. Licata  H. Nguyen  S. Pisupati
S. Gluf  S. Musall  D. Nunes Raposo  H. Read

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—that is, 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 decisions is critical for two reasons. First, it is essential for a complete understanding of sensory perception because real-world stimuli rarely activate a single sense in isolation. Therefore, understanding how the brain interprets incoming information requires understanding how the brain merges information from different senses. Second, it is likely of clinical importance: Several developmental abnormalities appear to be related to difficulties in integrating sensory information. For example, abnormalities in multisensory processing are a hallmark of subjects with autism spectrum disorder. 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 (Raposo et al., J Neurosci 32: 3726 [2012]; Sheppard et al., J Vis 13: 4 [2013]) In addition, we built on the foundation by measuring the responses of neurons in rodents engaged in the behavior, affording insight into the neural circuits underlying multisensory decisions (Raposo et al., Nature Neurosci 17: 1784 [2014]).

Microcircuits within Posterior Parietal Cortex That Support Decision-Making

M. Kaufman, A. Licata, L. Chartarifsky

There are two projects within this category. The goal of the first project is to gain a deeper understanding of the neural circuits that allow auditory, visual, and multisensory decision-making. This year, we began using an optogenetic strategy to manipulate neural activity and, thus, understand the causal role of different areas in decision-making. Technician Angela Licata led this project and presented data at the Society for Neuroscience Annual Meeting. After the conference, Matt Kaufman joined the project; the paper has now been submitted for publication.

The goal of the second project is to understand how the brain estimates the reliability of sensory stimuli and uses that estimate to guide decision-making. To reveal mechanisms that support this behavior, Lital Chartarifsky will use multisensory stimuli in which
information from the two modalities differs. These are called “conflict stimuli” and in Lital’s experiments, these will be auditory and visual stimuli that differ in their number. She will leverage a large body of human literature that has explored these behaviors and will bring them to a powerful rodent model to elucidate neural mechanisms. Collaborations with a neurosurgeon may provide an opportunity to bridge the gap between human and rodent decision-making.

Population Dynamics of Neurons during Decision-Making
M. Kaufman, F. Najafi

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 Kaufman is measuring the responses of 300 to 400 neurons simultaneously using two-photon imaging. This approach will revolutionize the kinds of questions about decision-making that we can address, especially when used in conjunction with emerging mathematical techniques for analysis. This year, Matt was supported by a grant from the Simons Collaboration on the Global Brain.

Farzaneh Najafi is, likewise, interested in population dynamics. She uses two-photon microscopy to image neural responses during decision-making in mice. She will focus on how excitatory and inhibitory neurons together contribute to evolving population responses. Her approach will include a consideration of the animal’s strategy, such as the degree to which it uses the outcome of previous trials to guide current decisions.

Both projects have benefited from technical support from the Albeanu lab, our neighbors in the Marks building and valued collaborators both at Cold Spring Harbor and at Columbia University.

Brainwide Macrocircuits That Support Decision-Making
S. Musall, S. Pisupati, O. Odoemene, H. Nguyen

The goal of this project is to understand how multiple neural areas make up brainwide macrocircuit circuits that support decision-making. The project has three key components.

The first component is led by Onyekachi Odoemene and will rely on inactivation methods to determine the role of multiple visual areas in transforming sensory information into signals that guide action. Onyekachi uses imaging methods to identify primary and secondary visual areas and then separately inactivates these and examines the effect on behavior. In the past year, he has fine-tuned these techniques and is now planning high-throughput experiments that put the techniques to use.

The second component is led by Simon Musall, a new postdoctoral fellow who received his Ph.D. from Universität Zürich. Simon will use a combination of wide-field and two-photon imaging to understand which neural areas are active during different moments of a perceptual decision. To do so, he has been developing new behaviors in mice. Research technician Hien Nguyen has provided support for this project, especially the behavioral component. She has expertise in training paradigms for mice gained from collaborations with others in the lab during her last few years here.

The final component is led by Sashank Pisupati, a new graduate student in the lab. Sashank will add to our understanding of decision circuits by systematically inactivating multiple brain areas in rats and examining the effects on unisensory decisions and multisensory integration. He will use the framework for optimal combination of stimuli (Ernst and Banks, Nature 415: 429 [2002]) to distinguish unisensory deficits from deficits in multisensory integration.

PUBLICATIONS


MECHANISMS OF NEURODEGENERATION AND MEMORY FORMATION

J. Dubnau  R. Borges Monroy  W. Li  M.F. Shih  L. Krug  L. Prazak

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 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, L. Krug, L. Prazak, J. Dubnau [in collaboration with M. Hammell and Y. Jin, CSHL]

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. We used mutations in Drosophila Argonaute 2 (dAgo2) to create a situation in which TEs are derepressed in the brain. Such mutants exhibit precocious TE elevation in young animals and exacerbated age-dependent derepression. This is accompanied by rapid age-dependent memory impairment and shortened life span. These findings support the hypothesis that protracted loss of TE silencing contributes to age-dependent decline in neuronal physiological function.

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). As a first foray into the role of TDP-43 in transposon control, we mined a series of deep sequencing data sets of protein–RNA interactions and of gene expression profiles and uncovered broad and extensive binding of TE transcripts to TDP-43 (Li et al., PLoS One 7: e44099 [2012]). We also found that association between TDP-43 and many of its TE targets is reduced in FTLD patients. Finally, we discovered that a large fraction of the TEs to which TDP-43 binds become derepressed in mouse TDP-43 disease models. On the basis of these findings, we propose the hypothesis that TE misregulation contributes to TDP-43-related neurodegenerative disease. This hypothesis is now being tested using a Drosophila model and examining post-mortem human tissue from ALS patients.

We have used an established Drosophila model of TDP-43 neurodegeneration to test the hypothesis that transposons contribute to the underlying pathology. On the basis of the bioinformatics analyses of sequencing studies in mouse, rat, and human (see above), we predict that aggregation pathology of the TDP-43 protein will disrupt the normal mechanisms of transposon silencing, leading to a storm of awakened transposons. Indeed, we find that overexpression of human TDP-43 in Drosophila leads to activation of gypsy transposon expression (L. Krug et al., in prep.). Interestingly, the TDP-43 pathology in glial cells shows more dramatic gypsy expression than manipulation solely in neurons. This is accompanied by apoptotic cell death, progressive defects in locomotion, and shortened life span. Moreover, using reporter systems, we have established that TDP-43 expression leads to a disruption of the normal cellular systems to silence transposons. Together, these findings support the idea that a storm of unregulated transposons contributes to TDP-43-mediated neurodegeneration. This idea is now being tested using post-mortem tissue from ALS and FTLD patients.
Age-Dependent Cognitive Decline and Retrotransposon Activation
L. Prazak and J. Dubnau

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 neuronal decline. 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 and stress on these cellular mechanisms, and (3) testing whether retrotransposon activation has a causal role in age-related cognitive impairment.

Neurogenetic Mechanisms of Olfactory Guided Behaviors
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.

Our current work on this topic is focused on forging links between gene function and neural circuitry and olfactory guided feeding behavior and olfactory memory. We are using cell-type-specific genomic approaches to identify memory-induced transcriptional changes within each of the neuronal cell types that form part of the circuit for olfactory memory processing in Drosophila.
The research in our lab aims to understand molecular events that initiate cellular signal transductions involved in neurotransmission in the mammalian brain with a scope to develop compounds with therapeutic value in the treatment of 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 on stimulation by voltage and/or neurotransmitters. Those ion channels regulate strength of neurotransmission, the fundamental process for neuronal communication. Dysfunction of the ion channels studied in our group is highly implicated in neurological disorders and diseases described above. The abnormal activation of the ion channels may be caused by a number of factors, including excessive transmission of neurotransmitters and point mutations in the ion channels that alter their functional properties. To understand functions of normal and abnormal ion channels, we use structural biology techniques including X-ray crystallography and single-particle electron cryomicroscopy to determine three-dimensional atomic structures of target ion channels and test structure-based mechanistic hypotheses by site-directed mutagenesis in combination with biochemical and biophysical techniques such as electrophysiology. In 2015, we continued to dissect various functional aspects of the major neurotransmitter receptor ion channel type, N-methyl-D-aspartate receptor (NMDAR).

Crystallographic Study of Antagonist-Bound GluN1/GluN2A Ligand-Binding Domain for Development of GluN2A-Specific Compounds

Development of antagonists against then hypothetical glutamate receptors by chemists in the 1970s was instrumental in identifying what we know as N-methyl-D-aspartate (NMDA) receptors today. Because a number of neurological diseases and disorders are caused by hyperactivation of NMDA receptors, there was an enormous degree of enthusiasm to further develop NMDA receptor antagonists for therapeutic purposes. This effort failed initially, mainly because of non-subtype-specific targeting of NMDA receptors. However, recent studies indicate that subtype-specific targeting of NMDA receptors is effective in treatment of treatment-resistant depression—rekindling enthusiasm for antagonizing NMDA receptor function, but in a subtype-specific manner.

Our recent study revealed the mechanism of antagonist recognition for the first time. The study showed that interaction between GluN2A Tyr730 and a phosphono group of an antagonist (Site-I) determines NMDAR specificity over non-NMDARs, whereas interaction of a hydrophobic group (e.g., phenanthrene ring in PPDA) of an antagonist with the hydrophobic pocket (Site-II) determines the subtype specificity within NMDARs (Fig. 1). Thus, we now focus on lead compounds containing both phosphono and hydrophobic groups such as SDZ220-040 (SDZ) and NVP-AAM077 (NVP) (Fig. 1), which show some degree of specificity toward GluN2A over GluN2B subtype. Here, we seek to pinpoint the molecular determinant for this moderate GluN2A specificity with scope for providing a strategy to design GluN2A-specific compounds with higher subtype specificity over NVP. To achieve our goal, we have initiated crystallographic studies of SDZ and NVP by soaking the GluN1/GluN2A ligand-binding domain (LBD) crystals in complex with glycine and glutamate against buffers containing SDZ (0.1 mM) and NVP (0.1 mM and 1 mM) as performed in our study of D-AP5 and PPDA above. We have solved the preliminary structures of SDZ and NVP complexes at 2.4 Å and 1.6 Å, respectively, by molecular replacement (MR) using.
the GluN1/GluN2A LBD structure split into D1 and D2 (upper and lower lobe of the bilobed structure).

Based on the structure, we are screening for compounds in silico to assess the likelihood of binding by molecular dynamics by the software in the Schrödinger suite in collaboration with Snyder’s group at Emory University. Specifically, we built the compounds with various linker carbon lengths and different halogens (Fig. 1; \( C_n \) and \( X \)) at pH 7.4 using the program epik and searching for energetically favorable conformations using confgen. The compounds are docked to the GluN1/GluN2A LBD structure by Glide using extra precision (XP) scoring algorithm to obtain the best scoring compounds during docking. The newly designed backbone of the compounds will be synthesized and tested for subtype specificity toward GluN1/GluN2A over GluN1/GluN2B NMDA receptors by electrophysiology.

**Engineering of Anti-NMDA Receptor Antibodies That Alter Ion Channel Functions**

Recently, our group isolated functional antibodies that bind GluN1/GluN2B NMDAR and attenuate ion channel activity by immunizing mouse with the intact GluN1/GluN2B NMDAR proteins at the CSHL antibody facility. Those antibodies give positive signal in ELISA (enzyme-linked immunosorbent assay) and no signal in western blot, indicating that they recognize protein fold rather than unstructured peptides. A size-exclusion chromatography analysis (Superdex200) of the mixture of the purified GluN1b/GluN2b amino-terminal domain (ATD) proteins and the IgGs shows a shift in retention time compared with GluN1b/GluN2b ATD with no IgG, indicating that they recognize GluN1/GluN2b NMDAR at ATD. Furthermore, we have cloned the Fv fragment of the IgGs and established an effective expression/purification method involving Brevibacillus. The size-exclusion chromatography analysis shows the retention shift with GluN1/GluN2b ATD mixed with Fv2 but not with GluN1/GluN2a ATD, confirming that the isolated Fv fragments bind specifically to GluN1/GluN2b NMDARs. The electrophysiological (two-electrode voltage clamp [TEVC]) analysis shows slow and partial (~40% and ~30%, respectively) inhibition of GluN1/GluN2b NMDAR by 1 \( \mu \)g/mL of the IgG and Fv, respectively. We are working toward measuring binding affinity by isothermal titration calorimetry (ITC) and surface plasmon resonance (SPR).

Currently, the extent of inhibition for the inhibitory antibody is rather modest, ~50% at a maximum concentration tested. To increase affinity and efficacy...
of our inhibitory antibodies above, we conducted affinity maturation using phage display at the CSHL phage facility. Thus, we will increase the length of CDR-L2 by four to seven randomized residues. Toward this end, we constructed affinity maturation libraries targeting CDR-L2 of the Fab. The affinity matured Fab fragments will be tested for inhibition of ion-channel activity of NMDA receptors.

Autoimmune Antibodies against NMDA Receptors

Anti-NMDA receptor encephalitis is a potentially lethal form of encephalitis that is caused by autoimmune antibodies against NMDA receptors. Patients with anti-NMDA receptor encephalitis show psychiatric and cognitive symptoms, including memory loss, hallucinations, and paranoia. The disorder progresses to severe neurological dysfunction, including seizures, dyskinesias, impaired consciousness, and autonomic instability, which frequently requires mechanical ventilation. The immunosuppressive therapies reduce the serum and CSF (cerebrospinal fluid) titers of anti-NMDA receptor antibodies; however, there is no specific treatment for anti-NMDA receptor encephalitis. In collaboration with the Children’s Hospital of Philadelphia (CHOP)/University of Pennsylvania and the Lankenau Institute for Medical Research, we are attempting to identify and isolate the anti-NMDA receptor antibodies.

PUBLICATIONS


We study the developmental assembly and functional operation of neural circuits in the neocortex of the mouse. The neocortex consists of a constellation of functional areas that form a representational map of the external (sensory, social) and internal (visceral, emotional) world. These areas are strategically interconnected into elaborate information processing networks that guide behavior. Our overarching hypothesis is that, at the cellular level, cortical processing streams and output channels are mediated by a large set of distinct glutamatergic pyramidal neuron types, and functional neural ensembles are regulated by a diverse set of γ-aminobutyric acid (GABA)ergic interneuron types. We use state-of-the-art genetic approaches to systematically target cell types and facilitate the application of a full set of modern techniques for exploring cortical circuits. Among GABAergic interneurons, the chandelier cell (ChC) is one of the most distinctive cell types that control pyramidal neuron firing at the axon initial segment. We are studying the developmental specification, activity-dependent circuit integration, and functional connectivity of ChCs, an entry point toward understanding a local circuit module. Regarding pyramidal neurons (PyNs), we are systematically characterizing the developmental origin, axon projection pattern, and input connectivity of multiple classes of pyramidal neuron types, focusing on the forelimb motor cortex. We combine a range of approaches that include genetic and viral engineering, genetic fate mapping, gene expression profiling, cellular imaging, electrophysiology, and behavior analysis. Recently, we began to integrate these studies in the context of the motor cortex control of forelimb movements.

Genetic Targeting of Pyramidal Neuron Subtypes in Mouse Neocortex

Major obstacles when studying the development and organization of neural circuits in the cerebral cortex are the stunning diversity of neuron types and a lack of comprehensive knowledge about their basic biology. Glutamatergic 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. We have begun to build a comprehensive genetic tool set for major PyN subtypes in the mouse. 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. We have already generated 12 knock-in driver lines that target restricted populations of corticothalamic, corticofugal, corticostriatal, and corticocortical PyNs.

Role of Intermediate Progenitors in the Specification of Cortical Pyramidal Neuron Subtypes

The progenitor cells that give rise to cortical PyNs mainly include radial glial cells (RGCs) and intermediate progenitor cells (IPCs) located in the embryonic cerebral ventricle wall. RGCs divide asymmetrically to generate neurons either directly or indirectly through IPCs, which divide symmetrically to produce pairs of PyNs. It remains unclear how progenitor types (e.g., RGCs, IPCs), their lineage progression, and timing of neurogenesis contribute to the specification of diverse PyN subtypes defined
by axon projection, connectivity, and physiology. In particular, the role of IPCs in the generation of PyNs is poorly understood. The T-domain transcription factor (Tbr2) is specifically expressed in cortical IPCs. We have generated an inducible Tbr2-CreER mouse driver, which allows comprehensive lineage tracing from IPCs, and have developed a novel genetic method to fate-map neurons according to their lineage and precise birth time. We have used the Tbr2-CreER driver to fate-map IPCs throughout embryogenesis. In addition to assessing the laminar position of PyN subtypes, axon projections are analyzed with a novel method using viral labeling of fate-mapped PyNs. Using retrograde virus along with our genetic driver and reporter lines allows us to restrict cell labeling by progenitor type, birth date, and projection target. With this method, we further elucidate the PyN subtypes born from IPCs throughout neurogenesis, using axon projection as a more descriptive definition of cell type than laminar location. Fate-mapping experiments revealed that IPCs sequentially gave rise to PyNs with distinct laminar patterns spanning multiple nonconsecutive layers with only a trend toward an inside-out sequence. This suggests that IPCs do not generate PyNs in a strictly inside-out manner. Rather, temporal cohorts of multiple fate-restricted IPCs simultaneously, as well as sequentially, generate PyN subtypes defined by their axon projection and laminar location. Ongoing experiments include an investigation of the PyNs that are generated by direct neurogenesis from RGCs, in contrast to those that are generated by IPCs, and the development of a novel genetic birth-dating strategy using the Tis21 gene, which is expressed in progenitors as they undergo neurogenic division. These findings begin to link progenitor type and time of neurogenesis to the specification of PyN subtypes.

The Progenitor Origin of ChCs

The diversity of GABAergic interneurons reflects a division of labor in regulating the balance, flexibility, and dynamic operations of cortical circuits, but the developmental processes that generate these diverse cell types are not well understood. In particular, it is unknown whether multipotent progenitors progressively generate multiple cell types or fate-restricted progenitors generate specific cell types. The ChC is one of the most distinctive GABA types that innervates the axon initial segment of PyNs, and provides a more tractable experimental system. We have previously shown that a major cohort of ChCs is generated from Nkx2.1 progenitors in ventral medial ganglionic eminence (MGE) during late gestation. We use an intersectional approach to further define the progenitor types and their temporal progression throughout embryogenesis that give rise to ChCs, including laminar subtypes. By combining Nkx2.1-Flp and Ascl1- or Dlx1-CreER drivers and an intersection reporter, we distinguish MGE radial glia-like progenitors (RGCs, Nkx2.1+) and two types of intermediate progenitors (IPCs, Nkx2.1+/Ascl1+ or Nkx2.1+/Dlx1+). We found that, unlike most MGE-derived interneurons, ChC production and laminar deployment do not follow an inside-out pattern: Both L5/6 and L2 ChCs in somatosensory and frontal cortices are generated concurrently from mid to late gestation. Whereas Nkx2.1+ RGCs generate L5/6 ChCs during mid gestation, Nkx2.1+/Ascl1+ IPCs generate L2 ChCs during mid and, especially, late gestation, and L5/6 in late gestation. In contrast, Nkx2.1+/Dlx1+ IPCs generate few ChCs. Surprisingly, fate-restricted progenitors emerge as early as E10, but remain latent for several days before ChC neurogenesis. Together these results implicate the role of fate-restricted progenitors in the generation of distinct cortical GABA interneurons.

Retinal Input and Callosal Neuron Axons Regulate Activity-Dependent Pruning of ChCs

Proper distribution and wiring of cortical inhibitory interneurons are crucial in regulating the balance of local circuitry and routing of cortical information flow. The ChCs innervate PyNs at their spike initiation site; thus, the proper distribution of ChCs may play a major role in shaping functional PyN ensembles. After their specification in the late embryonic medial ganglionic eminence, ChCs migrate with stereotyped route and schedule to arrive at designated cortical layers by the end of the first postnatal week. Surprisingly, we found that young ChCs then undergo massive apoptosis throughout the developing cortex between P7 and P14. In particular, the density of ChCs at the V1–V2 border region is reduced by half. The V1–V2 border receives input from callosal
neuron (CN) axons of the contralateral visual cortex. By blocking CN growth or activity using Kir2.1 or inhibitory DREADD, we found that contralateral CN activity among P7–P14 regulates the survival and density of ChCs at the border region. Further, ectopic CN axon projection induced by monocular enucleation at birth resulted in corresponding ectopic ChC elimination. Importantly, blocking retinal activity by monocular tetrodotoxin (TTX) injection among P7–P14 had no effect on CN axon projection, but reduced ChC pruning at the ipsilateral V1–V2 border. Together, these results suggest that the density of ChCs at the V1–V2 border is regulated by contralateral CN axons and retinal inputs. CNs receive input from the temporal retina that represents the central visual field and contribute to the seamless fusion of the left and right visual field. We hypothesize that activity-dependent elimination of ChCs at the V1–V2 border may contribute to the development of a fast bilateral signaling pathway among the CNs that correlate their discharges when stimulated by the same orientation stimulus. Current effort is directed toward understanding the role of ChCs in the interhemispheric callosal projection pathway that integrates the cortical representation of the central visual field.

Connectivity and Function of ChCs in a Medial Prefrontal Cortex Fear Circuit

The superficial layers of rodent medial prefrontal cortex comprise multiple information processing streams mediated by subsets of glutamatergic PyNs that project to distinct targets. Whether and how GABAergic interneurons regulate the segregation and communication among these intermingled PyN subnetworks remains unclear. We have discovered that a set of layer 2 (L2) GABAergic ChCs (or axo-axonic cells) selectively innervate PyNs projecting to the basal lateral amygdala (PBLA) over those targeting contralateral cortex (PCC) or striatum (PSTR). These ChCs are preferentially recruited by local and contralateral PCC reciprocal networks, and further receive long-range inputs from multiple cortical, subcortical, and modulatory sources. As ChCs selectively innervate PyN at their axon initial segments, these results reveal exquisite specificity in cortical local and global connectivity in which a distinct interneuron type mediates directional control between PyN subnetworks and, likely, the routing of long-range inputs. Chemical genetic suppression of PL L2 ChCs resulted in enhanced fear expression. Together these results suggest that prelimbic ChCs may integrate contextual, emotional, and motivational information to modify PBLA firing and achieve flexible regulation of fear expression.

Genetic Dissection of Motor Cortex Circuits That Control Forelimb Movement

The motor cortex has been implicated in the volitional control of forelimb movements, a rich set of behavioral skills that allow rodents to manipulate environment according to sensory inputs, internal goals, and experience. However, the underlying circuitry mechanisms remain largely unexplored. In particular, different types of glutamatergic PyNs (e.g., defined by their axon projection patterns) likely form multiple interareal processing streams and output channels that mediate sensorimotor integration and descending motor commands, but the cellular basis of these neural networks and output pathways is poorly understood. We have initiated a systematic genetic and viral targeting of PyNs based on their lineage, birth order, gene expression, and axon projection. Genetic access to these PyNs provides unprecedented opportunities to dissect motor cortex circuitry and output pathways. We are using multiple driver lines combined with anterograde and retrograde viral methods to examine the projection patterns and connectivity of several infragranular layer subpopulations. In addition, we are using optogenetic manipulation of these PyNs to explore whether they mediate specific components of forelimb movements in head-fixed awake mice.

PUBLICATIONS


Research


NEUROBIOLOGY OF COGNITION AND DECISION-MAKING

A. Kepecs  N. Bobrowski-Khoury  Q. Li  S. Ranade
E. Demir  M. Lorenc  T. Sikkens
B. Hangya  P. Masset  J.F. Sturgill
J. Hirokawa  H.J. Pi  C. Toschi
S.J. Li  J.L. Pie  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. Using a reductionist approach, we 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. Behavioral links to humans can also serve to identify behavioral dimensions that are predictive of disordered mental states, and our goal is to bridge our studies in animals with psychiatric disorders. 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 (i) the roles of uncertainty and confidence in decision-making, (ii) foraging decisions about whether to stay or to switch, (iii) the division of labor between different cell types in prefrontal cortex, (iv) how the cholinergic system supports sustained attention, and (v) social decisions that rely on shallow, stereotyped circuits. A unifying theme is the use of cell-type and pathway-specific perturbations to effect gain of function and loss of function for particular 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.

Confidence in Rats, Humans, and Statistics
B. Hangya, P. Masset [in collaboration with J. Sanders]

Confidence judgments (i.e., assessments about the quality of decisions) have been long studied as a central example of a metacognitive process unique to humans. More recently, we and others attempted to show that nonhuman animals are also capable of confidence judgments. Self-reports of confidence appear prima facie the only way to study confidence, yet they are neither necessary nor sufficient to establish what process led to the generation of the verbal report. Whether applied to humans or other animals, we are stuck with observable behavior. Previously, 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 statistical concept of “decision confidence” corresponds to the human notion of subjective confidence. Confidence can be framed as an objective statistical quantity—that is, an estimate of the probability that a chosen hypothesis was correct. Yet, 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. Based on 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 to make direct comparisons. We designed a perceptual discrimination task in which 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). Using several previously described models of choice and confidence, we tried 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 is, 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 these confidence-reporting tasks share the basic patterns of confidence that is predicted by statistical confidence. Based on these results, we are now in a position to use these quantitative measures of decision confidence in humans, through collaborations, with neuroimaging and genetic approaches. Moreover, by showing that a single confidence measure is applicable to humans and rodents, our results strengthen the case for using the rat as a model system for studying decision confidence.

**The Representational Content of Orbitofrontal Cortex during Outcome Anticipation**

J. Hirokawa, A. Vaughan, J.L. Pie

Orbitofrontal cortex (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. OFC neurons display a baffling diversity of behavioral correlates, and encode mixtures of sensory, motor, and other task-related variables in an apparently disordered manner. The question of what OFC and other frontal areas encode at the population level has been typically addressed either via model-based approach or dimensionality reduction. 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. We developed a model-free approach to understand the representational architecture of higher-order cortical areas, and use this method to reveal discrete functional groups of OFC neurons that encode distinct, model-based decision variables.

To address this, we trained rats in a task that combines two classic decision paradigms, one based on sensory uncertainty (perceptual decision) and one based on reward uncertainty (value-based decision) to engage OFC. Importantly, this task enabled us to probe the response profiles of each neuron across a large number of task contingencies. We then applied spectral clustering to these response profiles, and identified groups of OFC neurons showing stereotyped response profiles in which each group encodes an unmixed or categorical representation. Last, we found that these representational categories map onto distinct model-based decision variables, such as reward size, decision confidence, and integrated value, that serve as a sparse basis for a normative model of the behavioral task at hand. This analysis revealed nonrandom, structured representations of these decision variables in OFC. We propose that the representations in frontal cortex are, thus, fundamentally similar to the coding strategies used in sensory cortex in that they form sparse, overcomplete representations that are aligned to the natural statistics of the world—in this case, spanning the space of decision variables required for behavior.

**Impact of Drugs of Abuse on Decision-Making and OFC**

J.L. Pie, J. Hirokawa

Deficits in decision-making constitute a core disruption in drug abuse and addiction. OFC has emerged
as one important region for 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 a result of defects in integration of decision confidence and reward value in the decision-making process. Specifically, we aimed to identify the impact of a morphine self-administration protocol on optimal decision-making in the reward-biased two-alternative forced choice (2AFC) task.

We trained rats in the 2AFC task and exposed them to ~2 weeks of morphine self-administration followed by 2 weeks of withdrawal. These rats reliably evoked morphine self-administration. Following withdrawal, we tested for disruption of decision-making processes as deviations in two types of choice biases: reward-size bias that changes choices modulated the propensity to choose toward the larger reward, and previous outcome bias that generated changes in choice behavior based on the immediately preceding outcome. We observed distinct changes in choice bias: Reward-size bias was significantly reduced, whereas previous outcome bias was not, by morphine exposure. This result suggests that chronic morphine administration alters decision-making selectively, disrupting either a circuit- or cell-type-specific representation of choice bias. Based on 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.

Dopamine Prediction Errors and Confidence during Perceptual Decision-Making
A. Kepecs [in collaboration with A. Lak, University of Cambridge; and M. Sakagami, Tamagawa University]

Dopamine neurons signal reward prediction errors (i.e., the discrepancy between the value of the predicted and the received reward). This computation requires a prediction about the value of upcoming outcomes. In value-guided behavioral tasks, this prediction is based on the reinforcement history. Many types of decisions, on the other hand, are driven not only by previous outcomes but also immediate, often ambiguous, perception. For such decisions, predicting outcome values requires resolving the perceptual ambiguity and computing an instantaneous estimate of the probability of choice accuracy that reflects trial-by-trial fluctuations in perception. However, it has been unclear whether dopamine neurons are able to signal prediction errors incorporating such estimates. We used a computational model to show that dopamine neurons in monkeys performing a perceptual decision task comply with predictions of a reinforcement learning model extended with a belief state about the perceptual stimulus. As a feature of the belief state computation, this model generated trial-by-trial predictions of the probability of decision correctness, termed decision confidence. Similarly, we found that dopamine responses did not simply reflect the average value of the perceptually ambiguous stimuli but were predictive of the trial-to-trial fluctuations in decision confidence. These confidence-dependent dopamine responses emerged before the monkeys’ choice initiation and, thus, could potentially impact impending actions. Finally, by manipulating the reward size, we show that dopamine neurons reflect both the magnitude of the upcoming reward and the confidence in achieving it. Thus, dopamine responses convey the teaching signals that are also appropriate for learning perceptual decisions.

Quantitative Measures of Impulsive Choice and the Cost of Waiting in Mice
H.J. Pi, T. Sikkens

Impulsivity, a failure of inhibition in deliberative decision-making, is implicated in diverse psychiatric conditions and has been the focus of many clinical studies. Yet, the underlying neuronal mechanisms remain elusive. Whether a particular decision made too early is impulsive is difficult to determine because miscalculation of expected outcome or misestimation of time could lead to similar consequences. Therefore, as an initial step toward understanding the underlying neural circuits, we sought to develop a task that isolates the contribution of impulsivity to individual choices and separates it from reward valuation and
temporal estimation processes. We developed a novel cued lick-withholding task for mice and used a computational model to identify distinct behavioral processes contributing to each choice. Mice were trained to wait for a water reward delivered with uncertain temporal delays, and successful withholding of licking was rewarded. To dissociate impulsivity from temporal and reward expectations, we introduced three trial types with distinct waiting and reward-size contingencies that were cued by an auditory signal. We found that the probability of a correct response and withholding duration was low at the beginning of each session when mice were thirsty and progressively increased as mice became satiated, but these dynamics varied across trial types. To define a trial-to-trial measure of impulsivity, we designed a computational model based on a drift-diffusion process in which the cost of waiting is accumulated in proportion to the reward value, and a decision is made when a threshold is reached. The results captured the core observation that impulsivity negatively modulates waiting time. Together, our novel behavioral paradigm and computational modeling provide a means to disentangle the contribution of impulsivity from the reward valuation and time estimation in impulsive choice behavior.

Circuit and Behavioral Functions of Cortical Chandelier Neurons
Q. Li [in collaboration with Z.J. Huang, CSHL]

Chandelier cells (ChCs) are perhaps the most unique GABAergic interneurons in cortex and have fascinated neuroscientists for a long time. Their distinctive axonal morphology makes them look like an old-fashioned chandelier. ChCs “exclusively” innervate the axon initial segment of excitatory pyramidal neurons (PyNs), the site of action potential generation. Consequently, they have been thought to function as “veto cells,” suppressing spike initiation in PyNs. This has been called into question by in vitro studies showing that ChCs can paradoxically be excitatory. Until recently, it was not possible to study these neurons in the intact brain and, therefore, even elementary questions about function, such as whether they are excitatory or inhibitory, remained unanswered. Josh Huang’s lab (CSHL) successfully developed a novel genetic mouse line enabling the reliable targeting of ChCs, which was a game changer for the field. This will enable us, for the first time, to probe both the circuit impact and behavioral function of ChCs in behaving mice. We aim to provide the first direct evidence to address basic but crucial questions about how ChCs impact the circuit and when are they recruited during behavior. First, we will label ChCs in the medial prefrontal cortex (mPFC) with ChR2 to make them activatable. Then, we will use combined electrophysiological and optogenetic techniques to identify and manipulate the intracortical circuit between ChCs and postsynaptic PyNs in vivo.

Cortical VIP Interneurons, Disinhibitory Control, and Reinforcement Learning
H.J. Pi, B. Hangya [in collaboration with Z.J. Huang, CSHL; and B. Rozsa, KOKI, Hungary]

We are interested in understanding how the diversity of cortical inhibitory interneurons underlies distinct neural circuit dynamics that direct perception and behavior. We have recently identified a disinhibitory cortical circuit motif that appears to be a conduit for fast neuromodulatory action in cortex. This circuit is controlled by a class of inhibitory interneurons that express vasoactive intestinal polypeptides (VIPs), which inhibit other interneurons and thereby disinhibit a subpopulation of principal neurons. VIP neurons express ionotropic nicotinic acetylcholine and serotonergic 5-HT₃A receptors. Functionally, we showed that VIP interneurons in the auditory cortex are recruited in response to specific reinforcement signals such as reward and punishment. Although these observations raise the possibility that VIP neurons transform neuromodulatory signals to cortical output, the exact neuromodulatory signals that are involved remain unknown.

What are the mechanisms by which VIP neurons are recruited at specific moments in behavior? To identify which neuromodulatory systems recruit a VIP-controlled disinhibitory circuit, we took advantage of a rabies virus–assisted retrograde tracing system that fluoresceintly labels only monosynaptic input neurons. We identified direct projections from cholinergic neurons in the basal forebrain (BF) and serotonergic neurons in the raphe nuclei. Moreover, it is known that VIP neurons express ionotropic nicotinic acetylcholine and serotonergic 5-HT₃A receptors. Based on these results, we are pursuing the hypothesis that VIP neurons transform fast neuromodulatory signals into cortical disinhibitory output.
To explore the generality of these observations across cortex regions, we are collaborating with B. Rozsa (KOKI, Hungarian Academy of Sciences), who developed a state-of-the-art three-dimensional (3D) random access acousto-optic deflector (AOD) two-photon imaging system that allows recordings of the sparse VIP population across large volumes (~600 × 600 × 800 μm). In preliminary data, we observed that most VIP neurons are activated by reward and punishment across multiple cortical regions, suggesting that their behavioral recruitment has a cortex-wide function in reinforcement learning.

Neural Representation of Social Decisions and Rewards
E. Demir, N. Bobrowski-Khoury, C. Toschi [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, which have proven 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 other’s 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.

Male mice deposit scents in the environment that elicit attraction behavior in females. One component of these scents is darcin, a mouse urinary protein. Darcin is sufficient to induce innate attraction in sexually receptive female mice and also acts as an unconditioned stimulus in associative learning paradigms. We have identified a neural circuit that extends from the vomeronasal organ to the medial amygdala and mediates the innate response to darcin. Genetic silencing of either accessory olfactory bulb or the medial amygdala eliminated the innate attraction to darcin. Optical reactivation of darcin-activated medial amygdala neurons elicited attraction behavior.

We are also interested in understanding 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 the study of 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-offs 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.

Role of Cholinergic Basal Forebrain in Attention and Learning
B. Hangya, S. Ranade, M. Lorenc, S.J. Li, J.F. Sturgill

The cholinergic basal forebrain (CBF) constitutes a major but poorly understood neuromodulatory system important 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 BF cholinergic neurons. Yet, despite the association of CBF 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 CBF can have strong and confusingly diverse effects on downstream targets and behavior. However, their precise behavioral function is poorly understood because identified cholinergic neurons have never been recorded during behavior.

To overcome these challenges, we combine optogenetic and electrophysiological approaches to record during behavior from identified cholinergic projection neurons in two distinct nuclei of nucleus basalis (NB): the caudal region projecting to auditory cortex and the horizontal diagonal band that projects to prefrontal cortex. We have successfully developed visual and
auditory versions of sustained attention tasks for mice. We found that a non-CBF population—but not cholinergic neurons—were correlated with trial-to-trial measures of attention. Surprisingly, cholinergic neurons responded to reward and punishment with unusual speed and precision (18 ± 3 msec). Cholinergic responses were scaled by the unexpectedness of reinforcement and were highly similar across neurons and two nuclei innervating distinct cortical areas. These results reveal that the cholinergic system broadcasts a rapid and precisely timed reinforcement signal, supporting fast cortical activation and plasticity.

A Receptor Complementation Strategy to Overcome Tropism in Retrograde Targeting of Neurons

S.J. Li

Retrogradely transported neurotropic viruses enable targeting neurons based on their long-range projections and have, thus, become indispensable tools for linking neural connectivity with function. A major limitation of viral techniques is that they rely on cell-type-specific molecules for uptake and transport (viral tropism). As a result, viruses fail to infect variable subsets of neurons depending on the complement of surface receptors expressed. Here, we report a strategy to overcome this problem by enhancing the expression of receptors for the retrograde virus of interest, in this case, canine adenovirus type 2 (CAV-2). We designed adeno-associated virus (AAV) vectors, which cause cells in the source region to express the coxsackievirus and adenovirus receptor (CAR) throughout candidate projection neurons’ axonal arbors. Enhancement of CAR expression greatly increased retrograde labeling rates with CAV-2, which we showed in several long-range projections in both rats and mice. Our results show a strategy to abrogate endogenous viral tropism, and thereby facilitate efficient retrograde targeting for functional analysis of neural circuits.

PUBLICATIONS


In Press

Our laboratory works on theories of neural computation. Our overall strategy is to use methods developed in mathematics, physics, machine learning, computer science, and statistics to build experimentally testable models of neural networks and their functions. In most cases, we base our theories on what is known about particular biological systems; however, given that the principles of brain function remain unclear, in many cases, we resort to building machine learning theories. This means that we formulate the problems solved by the brain in a mathematically rigorous fashion and hypothesize how an engineer would solve the problem. We then use these solutions to form experimentally testable predictions. Testing these predictions in collaboration with our experimental colleagues helps us refine our models. For example, we are interested in understanding how connectivity is established in the brain. We have proposed several theories that may determine the rules of making connections between neurons based on a limited set of instructions contained in the genome. These theories address several levels of organization, including computational, biological, engineering, and evolutionary. Our theories may explain the differences between connectivities in normal and abnormal brain circuits. We are also interested in understanding the principles of perceptual invariance—that is, how can sensory systems represent objects in the environment despite substantial variations in intensity and background? Visual percepts, for example, retain basic features, such as perceived shape and color composition, despite variable luminance, spectral composition, scale, and position of the stimuli. Although we study the question of perceptual invariance in application to well-defined problems, we believe that the principles that we will uncover may generalize across sensory modalities. Finally, we are pursuing the question of how modern theories of machine learning and artificial intelligence can apply to brain function. Although reinforcement learning, deep learning, long short-term memory networks, etc., are successful in solving a variety of artificial intelligence problems, their mapping onto brain circuits remains unclear. We attempt to bring these systems closer to satisfying the constraints imposed by biology. We hope that the convergence of machine-learning theories and biology will help us learn more about brain function.

**Primacy Coding in the Olfactory System**

This work was done in collaboration with D. Rinberg (NYU).

Humans can identify visual objects independently of view angle and lighting, words independently of volume and pitch, and smells independently of concentration. The computational principles underlying invariant object recognition remain mostly unknown. Here we propose that, in olfaction, a small and relatively stable set made of the earliest activated receptors forms a code for concentration-invariant odor identity. One prediction of this “primacy coding” scheme is that decisions based on odor identity can be made solely using early odor-evoked neural activity. Using an optogenetic masking paradigm, we define the sensory integration time necessary for odor identification and show that animals can use information occurring <100 msec after inhalation onset to identify odors. Using multielectrode array recordings of odor responses in the olfactory bulb, we find that concentration-invariant units respond earliest and at latencies that are within this behaviorally defined time window. We propose a computational model showing how such a code can be read by neural circuits of the olfactory system.

**Primacy Theory and the Evolution of Olfactory Receptor Repertoire**

This work was done in collaboration with D. Rinberg (NYU).

Sensory systems are constantly facing the problem of computing the stimulus identity, which is invariant with respect to several features. In the olfactory system, for example, odorant percepts have to retain
their identity despite substantial variations in concentration, timing, and background. How can the olfactory system robustly represent odorant identity despite variable stimulus intensity? We study a novel strategy for the encoding of intensity-invariant stimulus identity, which is based on representing relative rather than absolute values of the olfactory receptor responses. In particular, we propose that the identities of the strongest responding olfactory receptors represent odorant identity. Such an encoding imposes constraints on the affinities of the olfactory receptor ensemble. Human olfactory receptor family, for example, includes about 350 intact genes, making it the largest gene family in the genome. In this project, we develop statistical tests that should reveal the signatures of primacy coding in the responses, sequences, and connectivity of identifiable olfactory receptors. We show that primacy coding combined with the low (<350) dimensionality of the olfactory sensory space leads to formation of specific correlations in the olfactory receptor affinities. In particular, receptors that do not belong to a primacy set (i.e., the set of the strongest responding receptors) of at least one odorant are expected to be eliminated from the receptor repertoire. This finding suggests the presence of specific correlations in the responses on olfactory receptors to odorants. We find that such correlations are indeed present in the responses of Drosophila olfactory receptors. It also suggests correlations in connectivity between olfactory bulb/antennal lobe and piriform cortex/mushroom body. Overall, our findings suggest that the low-dimensional nature of the olfactory sensory space combined with the requirement to robustly represent odorant identity leads to a specific evolutionary pressure on the ensemble of olfactory receptors. This evolutionary model provides testable experimental predictions for the olfactory receptor responses and connectivity.

A Computational Framework for Converting High-Throughput DNA-Sequencing Data into Neural Circuit Connectivity

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

There is growing interest in determining the connectivity of neural circuits—the “connectome”—at single-neuron resolution. Most approaches to circuit mapping rely on either microscopy or physiology, but these approaches have very limited throughput. We have recently proposed BOINC (Barcoding of Individual Neuronal Connectivity), a radically different approach to connectivity mapping based on high-throughput DNA sequencing. In this project, we develop the set of computational algorithms that serve to convert sequencing data into neural connectivity. We used our computational pipeline on the results of proof-of-principle experiments, illustrating an implementation of BOINC based on pseudorabies virus (PRV). PRV is capable of traversing individual synapses and carrying DNA barcodes from one cell to another. Using this high-throughput sequencing data, we obtained a 456 × 486 connectivity matrix between putative neurons. An inexpensive high-throughput technique for establishing circuit connectivity at single neuron resolution would represent a major advance in neuroscience.
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 and imaging along with molecular genetic, optogenetic, and chemogenetic methodologies to probe and manipulate the function of specific neural circuits in the rodent brain, and determine their role in behaviors such as fear regulation, anxiety, reward- and motivation-related behaviors, and autism-related behaviors. We are currently undertaking the following major lines of research.

The Role of the Amygdala Circuitry in Fear Regulation and Anxiety

Our recent studies show that the central amygdala (CeA) has a key role in learning and expression of defensive responses to threats. In particular, our studies indicate that somatostatin-expressing (SOM+) neurons in the lateral division of the central amygdala (CeL) are essential for the acquisition and recall of conditioned freezing behavior—which has been used as an index of defensive response in laboratory animals—during Pavlovian fear conditioning. However, how exactly these neurons participate in fear conditioning and whether they contribute to the generation of defensive responses other than freezing remain unknown. More recently, by using fiber-optic photometry combined with optogenetic and molecular techniques in behaving mice, we show that SOM+ CeL neurons are activated by threat-predicting sensory cues following fear conditioning, and that activation of these neurons suppresses ongoing actions and converts an active defensive behavior to a passive response. Furthermore, inhibition of these neurons using optogenetic or molecular methods promotes active defensive behaviors. Our results provide the first in vivo evidence that SOM+ neurons represent a CeL population that acquires learning-dependent sensory responsiveness during fear conditioning and furthermore reveal an important role of these neurons in gating passive versus active defensive behaviors in animals confronted with threat.

Brain Circuits Involved in Susceptibility and Resilience to Stress

Some individuals are resilient, whereas others succumb to despair in repeated stressful situations. The neurobiological mechanisms underlying such divergent behavioral responses remain unclear. In a recent study, we used an automated method for mapping neuronal activity in search of signatures of stress responses in the entire mouse brain. We used serial two-photon tomography to detect expression of c-FosGFP—a marker of neuronal activation—in c-fosGFP transgenic mice subjected to the learned helplessness (LH) procedure, a widely used model of stress-induced depression-like phenotype in laboratory animals. We found that mice showing “helpless” behavior had an overall brain-wide reduction in the level of neuronal activation compared with mice showing “resilient” behavior, with the exception of a few brain areas, including the locus coeruleus, that were more activated in the helpless mice. In addition, the helpless mice showed a strong trend of having higher similarity in whole brain activity profile among individuals, suggesting that helplessness is represented by a more stereotypic brain-wide activation pattern. This latter effect was confirmed in rats.
subjected to the LH procedure, using 2-deoxy-2\(^{18}\)F\] fluoro-d-glucose positron emission tomography to assess neural activity. Our findings reveal distinct brain activity markings that correlate with adaptive and maladaptive behavioral responses to stress and provide a framework for further studies investigating the contribution of specific brain regions to maladaptive stress responses.

The Role of the Thalamic Reticular Nucleus Circuitry in Sensory Processing, Attention, and Cognitive Deficits Related to Schizophrenia

Selective processing of behaviorally relevant sensory inputs against irrelevant sensory inputs is a fundamental cognitive function whose impairment has been implicated in major psychiatric disorders. It is known that the thalamic reticular nucleus (TRN) gates sensory information en route to the cortex, but the underlying mechanisms remain unclear. In a recent study, we found that deficiency of the \textit{Erbb4}, a gene linked to schizophrenia, in somatostatin-expressing TRN neurons markedly alters behaviors that are dependent on sensory selection. Whereas the performance of the \textit{Erbb4}-deficient mice in identifying targets from distractors was improved, their ability to switch attention between conflicting sensory cues was impaired. These behavioral changes were mediated by an enhanced cortical drive onto the TRN that promotes the TRN-mediated cortical feedback inhibition of thalamic neurons. Our results uncover a previously unknown role of ErbB4 in regulating cortico-TRN-thalamic circuit function. We propose that ErbB4 sets the sensitivity of the TRN to cortical inputs at levels that can support sensory selection while allowing behavioral flexibility.

The Role of the Basal Ganglia–Lateral Habenula Circuitry in Reward Processing and Behaviors Related to Depression

Recent studies indicate that the lateral habenula (LHb) and the habenula-projecting globus pallidus (GPh) constitute a critical component of the brain reward circuitry. Neurons in the GPh–LHb pathway convey negative and positive motivational signals. In particular, both GPh and LHb neurons increase activity in response to unpleasant stimuli, and activation of these neurons induces aversive responses, at least in part through suppression of midbrain dopamine neurons. These observations point to a notable role of the GPh–LHb circuit in negative motivational control. On the other hand, neurons in the GPh–LHb circuit decrease their firing when experiencing pleasant events, although the functional significance of this response is unclear. Further, it remains unknown how such positive, as well as negative, motivational signals in the GPh–LHb circuit participate in more complex behavior—such as decision-making in the face of potential reward and punishment—and how these signals are regulated by other structures within the brain reward circuitry. Bridging these knowledge gaps will have important clinical implications for improved treatments, as deficits in reward-related processes are the hallmark of mood- or motivation-related disorders, including depression.

To address these questions, we are investigating the role of the motivational signals in the GPh in decision-making, and determining how GPh function is controlled by the striatum, a major source of inputs to the GPh. Our study indicates that neurons in the GPh constitute a distinct basal ganglia output nucleus that mediates the evaluation of action outcomes. We found in a classical conditioning task that individual mouse GPh neurons bidirectionally encoded whether an outcome was better or worse than expected. Mimicking these evaluation signals with optogenetic inhibition or excitation was sufficient to reinforce or discourage actions in a decision-making task. Moreover, cell-type-specific synaptic manipulations revealed that the inhibitory and excitatory inputs to the GPh were necessary for mice to appropriately evaluate positive and negative feedback, respectively. Finally, using rabies virus–assisted monosynaptic tracing, we discovered that the GPh is embedded in a basal ganglia circuitry wherein it receives inhibitory input from both striosomal and matrix compartments of the striatum and excitatory input from the “limbic” regions of the subthalamic nucleus. Our results provide the first direct evidence that information about the selection and evaluation of actions is channeled through distinct sets of basal ganglia circuits, with the GPh representing a key locus in which information of opposing valence is
integrated to determine whether action outcomes are better or worse than expected.

**PUBLICATIONS**


In Press
INTEGRATIVE SYSTEMS NEUROBIOLOGY

We study complex neurobiological systems using a combination of experimental and computational approaches. The primary area of experimental work in our laboratory continues to be the Mouse Brain Architecture (MBA) project, with a goal to generate a mesoscale connectivity map. The project has recently reached a major landmark, completing the acquisition of a full data set with one anterograde tracer (http://mouse.brainarchitecture.org/). Our neuroinformatics research involves the development of analytical tools and informatics infrastructures to process, analyze, and integrate large volumes of neurobiological data in various brain connectivity projects.

In collaborative studies, we apply the methods developed for the MBA project to other mouse strains, zebra finch, marmoset, macaque monkey, and human; here we highlight only a few. We completed a joint study with Josh Huang at CSHL (supported by the Simons Foundation) to obtain a brain-wide census of $\gamma$-aminobutyric acid (GABA)ergic interneurons in the mouse brain during development and in genetic models of autism, and we released all 140 whole-brain image data sets to the public on a dedicated portal (http://mouse.brainarchitecture.org/cellcounts/).

We are in the second year of collaboration with Pavel Osten at CSHL (funded by the National Institutes of Health [NIH] through the BRAIN Initiative program) to obtain accurate whole-brain census of neuron classes in cell type–specific reporter mice and develop neuroinformatics infrastructure to analyze and integrate such data. We have already posted the first dozen whole-brain image data sets on a newly developed dedicated portal (http://mouse.brainarchitecture.org/celltypes/). On a separate BRAINI grant (also in its second year), we have joined a team effort led by Sacha Nelson (Brandeis University) to complete a cross-species study of neuronal cell types in transgenic strains of rats and mice.

We have recently started collaborating with Professor Jeff Lichtman at Harvard University, who pioneered serial electron microscopy (EM) reconstruction techniques. We use complementary high-resolution imaging modalities to compare and contrast the growth of retinal ganglion cell axons (that form the optic nerve) in the situations in which accurate pathfinding occurs (the embryonic mammalian) with the situation in which it fails (regeneration in the adult mammalian optic nerve). The hope is that insight gained in this study will help aid optic nerve repair and restore vision in many patients who lose their sight to diseases of the eye that injure retinal ganglion cells.

I also collaborate with Professor Yoshio Okada at Massachusetts General Hospital to develop sonoelectric tomography, a next-generation noninvasive whole-brain functional imaging method that combines scalp electroencephalography (EEG) to record the electrophysiological signal with millisecond timing precision and ultrasound to encode brain location with millimeter resolution.

I am a distinguished (visiting) professor at the Indian Institute of Technology (IIT), Madras, where I initiated a Center for Computational Brain Research and organized a conference on Computational Brain Research, and collaborate with a team at the Department of Computer Science and Engineering. Last summer, our lab at CSHL hosted two IIT Madras computer science doctoral candidates for a rotation to further this collaborative effort.

Laboratory Personnel

Continuing in our laboratory in 2015 are Sandra Michelsen (Administrator), Alex Tolpygo (Laboratory Manager), Kevin Weber (Laboratory Technician), Stephen Savoia (Laboratory Technician), Angeliki Field-Pollatou (Visiting Graduate Student), Daniel Ferrante (Computational Science Manager), and Ferenc Mechler (Computational Science Analyst). Nicholas Baltera, Neil Franciotti (Laboratory Technicians), and Gregor Havkin (Computational Science Manager) have recently left our lab in pursuit of a
career in further education and industry, respectively. James Mulhern, Michael Weber (Laboratory Technicians), and Sotirios Raptis (Computational Science Developer) have joined during the year.

**MBA Project**

The MBA project is now in its sixth year. The project aims to construct a comprehensive mesoscale wiring map of the adult mouse brain. We use classical neuroanatomical approaches scaled to a high-throughput data acquisition pipeline. Using stereotaxic targeting, we microinject neurotracer substances (two anterograde or two retrograde) in one of the 380 predetermined sites systematically mapped on a three-dimensional (3-D) grid that covers an entire hemispheric brain volume. The sample brains are sectioned, histologically processed, and digitally scanned. We release to the public the registered high-resolution section images and metadata of project brains on the MBA Web portal (http://mouse.brainarchitecture.org/). The total number of brains on the portal now stands at more than 1000, representing 1300 injections. The published injections are summarized at the top in Figure 1, where each injected brain is represented by a colored dot mapped on the reference brain at the injection site. Figure 1 contains four injection overlays organized by tracer, from left to right: AAV (adeno-associated virus; red/green), \( N = 824 \) (in 559 brains), double injected brains used both green and red fluorescent constructs of the tracer; RV (rabies virus; green), \( N = 122 \); BDA (biotinylated dextran

![](image)

**Figure 1.** (Top) Published injections separately shown for the four tracers, from left to right: AAV, RV, BDA, CTB. (Bottom) The AAV data set summarized in minimum intensity projections (MIPs). Insets are zoomed examples.
amine; blue), \(N = 125\); and CTB (Cholera Toxin B Subunit; black), \(N = 203\). This year the project reached a landmark: we have completed data acquisition with the anterograde AAV tracer. The tiled composite image at the bottom of Figure 1 summarizes the AAV data set via minimum intensity projections (MIPs). Each brain is represented in three vertically stacked MIPs, one on each of the three cardinal planes. The zoomed examples show a red injection in somatosensory cortex (left) and a spatially offset red/green double injection in the subiculum (right). We assemble the mesoscale connectivity matrix from these sample brains after computational analyses that involve segmenting and tracing anterogradely labeled axonal projections and mapping them in a common reference space. With continued support from the Mathers Foundation, and with the current processing and publishing rate at approximately two brains per day, our expectation is to be able to complete data acquisition with a second tracer, the retrograde tracer CTB, in the forthcoming year. The retrograde connectome will be novel and will naturally complement the AAV data set.

In the computational domain, we have a number of ongoing projects working on cell/process detection and segmentation, most involving collaboration with outside experts. We have been working with the team of Professor Yusu Wang at Ohio State University to analyze the mesoscale circuit structure of the AAV connectivity data set using methods from computational topology and geometry (Morse theory and persistent homology). This approach to segmenting and tracing anterograde axonal projections is robust to noise and gaps in data and retains global shape information, which is lost in the connectivity matrix derived from the same data in conventional graph-theoretical analysis. Figure 2A shows a sample result. At CSHL, we have started doing segmentation and reconstruction of projection fields of fluorescently labeled anterograde

![Figure 2.](image)

Figure 2. (A) Skeletonization of anterograde tracer labeled motocortical axon projections. (B) Sample of Nissl-stained neurons from a scanned coronal section of an MBA mouse brain. (C) The detected and segmented somata in the same sample. (D) Detected retinal ganglion cell (RGC) axon terminals labeled by ocular injection of anterograde tracer in representative coronal sections. (E) 3D reconstruction of the segmented monosynaptic target fields of the right optic nerve (IIN). Grid scale, 2 mm. (F) Fluorescent scan of a coronal section from a SOM-Cre:H2B-green fluorescent protein (GFP) mouse model of autism spectrum disorders from the Huang lab; crosses overlaying cells mark the detected GFP-labeled nuclei.
projections (Fig. 2B,C). In collaboration with computer scientists at HHI at Janelia Farm and IIT, Madras, we are applying methods from computer vision and machine learning to develop computational solutions to automatically perform image segmentation and cell detection in large image data sets. The approach is data driven, and solutions are tailored to features characterizing the data that depend on the imaging modality, such as Nissl-stained cell bodies in bright-field images (Fig. 2D,E) versus neurons whose nuclei were fluorescently labeled (Fig. 2F). In an additional effort, the Madras team created a proofreading platform for manual correction of autosegmented data to annotate several whole brains. These will be used as high-quality ground truth data sets that will serve the training of machine-learning algorithms under development.

A Mesoscale Circuit Map of the Marmoset Brain

We have major ongoing collaborations with Marcello Rosa (Monash University, Australia) and Hideyuki Okano (RIKEN, Japan) on a project to develop the mesoscale circuit map and its first digital atlas of the marmoset brain. Our lab’s sister installation at RIKEN Brain Science Institute (Japan), where I am a senior visiting researcher, came online at the end of summer 2015. The RIKEN pipeline is an adaptation of the MBA pipeline technology to the needs of the larger brains of the New World primates. We anticipate that initially 25 brains a year will be processed (tracer-injected, sectioned, stained, scanned), each resulting in a Web-published whole marmoset brain data set.

The Cavity Method for Phase Transitions in Sparse Reconstruction and Variable Selection Algorithms

P. Mitra [in collaboration with A. Sengupta, Rutgers University]

This collaboration (in its second year funded by the National Science Foundation [NSF]) uses advanced analysis methods from statistical physics to gain insight on critical phenomena shown by sparse reconstruction of high-dimensional data. One area of application is retrieval of high-dimensional sparse correlated signals in compressed sensing; another is variable selection in machine learning. A common approach to both areas uses sparsity-inducing penalty functions that show sharp algorithmic phase transition boundaries between regimes of “good” and “bad” performance as the number of parameters tends to infinity. We use cavity mean field theory methods to characterize the performance of popular sparse reconstruction algorithms (e.g., LASSO or Basis Pursuit, and Elastic Net) around their phase transition boundaries.

PUBLICATIONS


In Press

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

P. Osten  G. Fitzgerald  A. Narasimhan  E. Szelenyi  K.U. Venkataraju
R. Munoz Castaneda  R. Palaniswamy  J. Taranda

Our work focuses on the study of anatomy and function of brain circuits in the mouse. We ask questions that probe the roles of brain circuits defined by their participation in specific behaviors, such as social behaviors, and we compare brain activation responses in normal (so called wild-type) mice and in mice engineered to carry genetic mutations linked to an increased risk of autism. The goal of our work is thus both to broaden our understanding of how brain circuits control complex behaviors and to identify brain circuit deficits that may serve as sensitive biomarkers for the development of novel treatments for autism.

Social Brain Circuitry in the Cntnap2 Knockout Mice

To determine the effect of the autism-linked Cntnap2 gene on brain circuits, we compared brain activation evoked by social behavior and object exploration in the Cntnap2 knockout (KO) mice and their wild-type littermates. Our study revealed that in the wild-type mice the social brain circuitry comprises more than 130 brain regions that are significantly more activated during social exploration compared with the exploration of an inanimate object. In contrast, the same comparison in the mutant mice identified only 60 brain regions significantly more activated by the social behavior test, revealing a dramatic brain circuit deficit in the Cntnap2 KO mouse model.

The phenotype in the Cntnap2 KO mice could be explained by two scenarios—first, it is possible that the social interaction evoked lower brain activation in the mutant mice, indicating “a lack of social interest” at the level of the social brain circuitry; second, it is also possible that the object exploration evoked higher brain activation in the mutant mice, thereby lowering the statistical difference between the two behaviors and indicating “a lack of preference between social and inanimate explorations.” The comparison of the whole-brain distribution of the c-fos+ cells after social behavior in the Cntnap2 KO and wild-type littermate mice revealed a largely unaffected response in the mutant mice. In contrast, the comparison of c-fos+ cell distribution after object exploration revealed higher activation in the Cntnap2 KO mice in brain regions normally activated during social behavior.

In conclusion, our data revealed normal social behavior–evoked brain activation and higher object exploration–evoked brain activation in the Cntnap2 KO mouse model of autism. These data suggest that the changes in the brain circuitry in the mutant mice may reflect behavioral changes seen in patients with autism, including more attention to nonsocial objects, such as gadgets, vehicles, and other objects of idiosyncratic “special interest.”

Abnormal Inhibition/Excitation Balance and Repetitive Behavior in the 16p11.2 df/+ Mice

A second model of autism that is a focus of study in our lab is the 16p11.2 df/+ mouse model, which was shown to have moderate brain size differences, including in the basal ganglia, suggesting a link to autism at a gross-anatomy level.

Our work with these mice revealed a striking finding of a partially penetrant epileptiform-like phenotype that correlates with an increase in repetitive behaviors and a deficit in sleep. First, we discovered that the convulsant drug pentylentetrazole (PTZ) at 30 mg/kg evoked clonic seizures in some but not all of the 16p11.2 df/+ mice, whereas the same PTZ dose failed to evoke seizures in their wild-type littermates. This seizure phenotype was stable, as nine out of 18 mice showed no seizure response in three repeated PTZ injections, whereas the other nine showed repeated clonic seizures evoked by each injection (Fig. 1A).

We also measured a variety of home-cage behaviors in this cohort of mice before the PTZ injections, which allowed us to examine whether there are any
behavioral differences between the 16p11.2 df/+ mice with normal seizure response and those with lower seizure threshold. Remarkably, this analysis revealed that although both groups of mice showed previously described hyperactivity compared with their wild-type littermates (Fig. 1B) (Horev et al. 2012), only the 16p11.2 df/+ mice with the lower seizure threshold showed increased grooming behavior (Fig. 1C) and decreased sleep (Fig. 1D).

In conclusion, our experiments with the 16p11.2 df/+ mice revealed for the first time a clear partial penetrance phenotype in a genetic mouse model of autism, replicating the well-documented partial penetrance of autism-linked mutations. In addition, these data support the hypothesis that abnormal excitation/inhibition balance and seizures often seen in children with autism may contribute to some behavioral phenotypes in autism.

Quantitative Cell Type–Based Analysis of the Mouse Brain

In addition to the above-described analysis of brain activation in genetic mouse models of autism, we are working on developing a quantitative cell-count-based understanding of whole-brain distribution of the various cell types that constitute the approximately 70 million neurons and approximately 30 million glia in the mouse brain. This work includes an analysis of whole-brain distribution of γ-amino butyric acid (GABA)ergic interneuron cell types, a collaborative project with the Josh Huang lab at CSHL, as well as analysis of cell distribution marked by the expression of imprinted genes (Grb10 and IGF2), and by the silencing of the X chromosomes.

PUBLICATIONS

NEURAL CIRCUITRY FOR SOCIAL COMMUNICATION

S. Shea  B. Cazakoff  B. Lau
D. Eckmeier  A. Stone

The broad goal of my laboratory’s research is to understand how sensory processing and neural plasticity in specific brain circuits facilitate communication and flexible behavior. We are revealing neural mechanisms that allow organisms to detect and recognize familiar individuals, gather information about their identity and social status, and select appropriate behaviors. Mice are capable of acquiring detailed profiles of their social partners from the smells and sounds experienced during their encounters. For example, they can discern another mouse’s sex, genetic identity, reproductive state, levels of distress, or sexual interest or even recently consumed foods. These details are indispensible for survival and mating success. In recent years, we have focused our attention on neural activity and circuitry in primary sensory brain areas that support communication. We are now 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 neural processing are flexible according to experience and behavioral state.

We are studying this problem for two reasons. First, we want to identify fundamental principles of how the brain adaptively controls complex behavior. In pursuing this goal, natural social behaviors are advantageous because they allow us to study neural circuits engaging the 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 (ASDs); 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 ASDs 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.

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 begins 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.

In 2014, we published a study of granule cell recorded in awake mice (Cazakoff et al., Nat Neurosci 17: 569 [2014]). In that paper, we reported the results of recordings made by Watson School of Biological Sciences (WSBS) student Brittany Cazakoff, postdoctoral fellow Dr. Billy Lau, and CSHL Undergraduate Research Program (URP) Kerensa Crump. The team developed methods to reliably and stably record granule cells from mice that were awake with their heads fixed, but running freely on a foam wheel and receiving water rewards from a lick tube. These 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.

This year Cazakoff achieved a major breakthrough by successfully extending her recordings of granule cells into mice that are not only awake, but actively learning new associations of odor stimuli with reward (water) and punishment (bitter taste). She has developed a behavioral paradigm in which the mouse learns that two new odors each signal either impending reward or the bitter tastant, respectively. They are also asked to learn that the odor associations have switched. The mice show that they have learned by licking when reward is signaled and withholding licks when the odor portends bitter taste. This learning develops in a time frame that allows us to monitor activity in individual granule cells throughout. These experiments reveal several very intriguing preliminary findings. First, the results suggest that the olfactory responses of the granule cells are far more intense when the animal is using its sense of smell to acquire rewards and avoid punishment. Second, granule cell activity also can reflect the delivery (or omission) of the water reward or the valence associated with a stimulus. These observations are consistent with our working hypothesis that, in behaving animals, these fascinating neurons are sensitive to a number of abstract task features that are independent of odor quality and identity.

Auditory Plasticity Is Impaired in a Mouse Model of Rett Syndrome
B. Lau, G. Ewall [in collaboration with J. Huang and K. Krishnan, CSHL]

Far beyond the limits of our hearing, in the ultrasound range, mice are continuously “speaking” (or vocalizing) to one another in a “language” that we have only just begun to understand. Many distinct vocalization types are produced by males, females, juveniles, and adults in a variety of behavioral situations. 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, before vision and full mobility, will occasionally become separated from the nest. This is stressful for them and they will, therefore, call out to their mothers with a very high frequency peep. New mothers develop sensitivity to these cries and respond by moving toward their source 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 ASD 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 that 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 resemble humans with Rett syndrome. It is also significant that this phenotype is a social communication deficit.

In work we completed previously, we showed that MeCP2 plays a critical role in maintenance and plasticity of the auditory cortex in support of this maternal behavior. Specifically, we observed that maternal experience triggers rewiring of the inhibitory networks of the auditory cortex, and that this process is disrupted when the gene MeCP2 is disabled. In a particularly exciting set of experiments, we were able to repair inhibitory function with genetic and pharmacological manipulations, thereby restoring maternal gathering behavior. These promising results focused our attention this year on how maternal experience and MeCP2 mutation affect the activity and function of the auditory cortex in an awake animal. We are particularly interested in the cortical network of inhibitory neurons that express a protein called parvalbumin (PV). This is because they are some of the neurons that are most dramatically affected by maternal experience, and because deletion of MeCP2 only in this minority of neurons is sufficient to disrupt pup care. Therefore, this year Dr. Billy Lau made neuronal recordings in
awake behaving females of both genotypes that differed in their maternal experience. The data show that PV+ neuron responses to playback of pup calls are suppressed in recently experienced mice. We speculate that this is a crucial trigger for neural plasticity. This suppression does not occur in MeCP2 mutants, but it is restored when we use a genetic method to repair their behavior. Our progress this year, therefore, strengthens our conclusion that MeCP2 mutations impair adult learning and plasticity through disruption of cortical PV+ networks.

**Neural Activity during Social Encounters**

D. Eckmeier

Another major advance for the laboratory this year was made by postdoctoral fellow Dennis Eckmeier. He successfully developed reliable methods for making stable recordings of individual neurons during actual social encounters between mice. His specific goal was to record from neurons in deep brain neuromodulatory centers during these encounters. Neurons that release neuromodulators, such as 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. As a first step, Dennis made recordings during mating encounters from the brain stem nucleus locus coeruleus (LC), which releases noradrenaline throughout the brain and affects sensory processing of olfactory cues (Fig. 1). Analysis of this rich data set is ongoing, but preliminary inspection suggests that there is dynamic modulation of LC activity during social behavior that would not have been predicted from previous recordings during nonsocial behavior. In the future, we intend to also examine the encoding of social information, such as body odors and vocalizations, in primary sensory structures. 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.

**Various Collaborative Projects**

S. Shea [in collaboration with N. Tonks, B. Li, and P. Osten, CSHL; R. Shansky, Northeastern University]

This year we also published the results of several collaborations with other laboratories at CSHL and Northeastern University. First, we showed with Nick Tonks’ laboratory that a small molecule inhibitor of

![Figure 1. Physiology during free behavior. (A) Overlaid spike traces from a single unit putatively in locus coeruleus (LC). Scale bar, 1 msec. (B) Video still frame taken from the recording. In the left half of the still, an angled mirror shows the side view of the mice. (C) Ninety seconds of data recorded from the cell during a period that included a 30-sec copulation event. The arrow shows the time of the still frame in B. (D) Histogram of firing rate data from C binned at 1-sec intervals.](image-url)
the protein phosphatase PTP1B is effective in alleviating behavioral and other symptoms in a mouse model of Rett syndrome. Second, we reported with Rebecca Shansky’s laboratory at Northeastern University our discovery of a novel learned fear response in female rats that we termed “darting,” motivating a reexamination of conventional definitions of fear in rodent models. Third, in collaboration with the Li and Osten laboratories, we analyzed whole brain activity patterns in mice that responded to stress and adversity positively (“resilient” mice) and mice that responded negatively (“learned helpless” mice). We described a characteristic pattern of differences across many brain regions that involved depressed activity in all brain regions with the exception of the tiny noradrenergic brain stem nucleus LC.

**PUBLICATIONS**


*In Press*

Our lab seeks to understand the mechanisms that shape and regulate differences in the brains of males and females. We couple genomic and molecular biology approaches with behavioral analyses to understand how neuronal identity is programmed by gene regulatory events, and how this identity imparts function within a neural circuit. We use innate social behaviors in mice as a readout for our molecular genetic manipulations. Males and females display different behavioral responses to the same social stimulus, such as the presence of an adult male intruder or the cries of pups. These behaviors are mediated by sexually dimorphic neural circuitry that develops under the control of the gonadal hormones estrogen and testosterone during a neonatal critical period. Manipulating hormone signaling during this critical period produces lasting effects on gene expression, brain wiring, and adult behavior.

Human males experience developmental testosterone surges, the intensity of which correlates with increased male-typical social behaviors and interests in childhood. Male mice similarly undergo a surge of testosterone at birth that rapidly subsides within the first few days of life. Our studies and those of others have found that in the brains of mice, it is the female sex hormone estrogen that is the primary mediator of the effects of neonatal testosterone. Estrogen is produced from testosterone by aromatase, which is expressed in discrete locations within the brain. Neonatal estrogen then acts to permanently establish the sex-specific neural circuitry that mediates behavior in the adult. Females given estrogen at birth display male-typical fighting behavior as adults with no additional hormone supplementation. Furthermore, neonatal estrogen is necessary and sufficient to produce sex-typical expression patterns of both its own receptor (estrogen receptor alpha, ERα), as well as the androgen receptor (AR), the cognate receptor of testosterone. Both of these receptors are required for correct displays of sex-specific behaviors such as mating, aggression, and maternal care. As with such behaviors, the actions of neonatal estrogen on gene expression persist into adult life; switching hormonal profiles to that of the opposite sex does not produce corresponding alterations in AR or ERα expression. Thus, the actions of neonatal estrogen determine the capacity of both genes and circuits to respond to hormones in adulthood. These long-lasting effects suggest that neonatal estrogen organizes the developing brain through an epigenetic mechanism whereby the transient signal at birth irreversibly modifies the chromatin state of genes. The nature of this mechanism, as well as the identity of the genes regulated by neonatal estrogen in the brain, is the focus of our current research program.

Many serious mental health disorders display a sex bias in incidence, age of onset, or presentation: Males are more likely to develop a neurodevelopmental disorder, such as autism, schizophrenia, or language delay, whereas adolescent-onset mood disorders, such as anxiety and depression, are more common in females. There is a clear need for a mechanistic understanding of the molecular events that underlie such sex biases. Our goal is to identify genes and genomic regions that impart sex specificity to neuronal function and to reveal general principles that underlie hormonal regulation of differential gene expression between the sexes. Understanding the molecular processes underlying sexual differentiation of the brain will therefore reveal new insights into the developmental trajectories of psychiatric disorders, ultimately leading to novel targets and treatments.

Dissecting Functional Subpopulations of Sexually Dimorphic Brain Regions

ERα plays a critical role in sexual differentiation of the brain; it is required for sex-specific behaviors in both males and females. Males constitutively null for ERα show severe deficits in mating behaviors and never display territorial aggression, indicating that the masculinizing effects of neonatal estrogen are exerted primarily through this receptor. However, as ERα is expressed in multiple brain regions, and throughout the body, it is unclear how different subpopulations of ERα neurons play roles in the circuitry that directs male behaviors. We have used a bipartite genetic
strategy to assess the distinct roles of excitatory and inhibitory ERα neurons in male-typical behaviors such as mating and territorial displays. We have deleted ERα in either excitatory neurons, using a vGlut2-Cre driver, or inhibitory neurons, using vGAT-Cre. Mutant males from both crosses have a strong reduction in mating behavior; the majority fails to complete a 30-min assay. Intriguingly, males null for ERα in vGAT+ inhibitory neurons display a striking deficit in territorial marking, an assay that assesses male-typical behavior in the absence of any social stimuli. Males mark a novel cage by scattering approximately 200 urine spots across the floor, whereas females always deposit their urine in large pools along the edges of the cage. Our mutant male mice display a feminized pattern of cage marking resembling that of wild-type females, suggesting that the inhibitory neurons that express ERα define an internal representation of “maleness.” We are currently working toward temporal control of ERα deletion, through the use of inducible Cre drivers developed in the lab of Z. Josh Huang. This approach will allow us to parse out the roles of this receptor in both the developmental organization and adult activation of sexually dimorphic neural circuitry.

Identification of Sex-Specific Gene Programs in the Brain

Male and female mice have overt differences in the structural and molecular organization of limbic brain areas such as the medial amygdala (MeA) and the bed nucleus of the stria terminalis (BNST)—regions critical to a wide variety of sex-specific behaviors. To understand how neonatal estrogen directs sexual differentiation of these brain regions, we are performing transcriptomic profiling of the MeA and BNST in male and female pups. To circumvent the massive heterogeneity of the mammalian brain, we are performing our RNA-seq experiments specifically in ERα neurons through the use of the RiboTag mouse line. This allele permits Cre-defined tagging of ribosomes, followed by purification of the affiliated translating messenger RNAs (mRNAs).

Our preliminary findings indicate that gene pathways associated with receptor recycling, vesicle transport, and synaptogenesis are enriched in male brains, whereas programs linked to the complement pathway, immune responses, and cell adhesion are more highly expressed in female samples. These results are consistent with the idea that neonatal estrogen directs the growth of a sex-specific circuitry in the male, whereas pruning of processes and neural apoptosis occurs in the female. We are continuing postsequencing analysis and validation of our differentially expressed gene profiles and are beginning to collect samples from adult males and females to determine how adult hormonal profiles alter the gene expression programs defined by neonatal estrogen. Future directions for this project include examining gene expression profiles in animals given exogenous hormone early in development, as well as in animals devoid of gonadal hormones.

Sex Differences in the Epigenome

Differences in innate behaviors between the sexes are the final readout of varying gene expression programs, and the nuclear chromatin machinery that establishes these genetic differences plays an essential role through the regulation of specific genes. Our lab is particularly interested in how chromatin architecture and the epigenome sculpt and enforce these gene expression differences between males and females. To gain access to the chromatin specifically from neurons expressing ERα in our brain regions of interest, we are using a protocol termed isolation of nuclei tagged in specific cell types (INTACT). This approach uses a genetic strategy to inducibly tag cell nuclei in a Cre-dependent fashion, thereby permitting preparation of chromatin from genetically defined cell types in the brains of male and female mice. Chromatin regulation is multilayered and we have decided to focus on two aspects of this process, specifically the extent of chromatin accessibility by various regulatory proteins and complexes and the covalent modifications of histones that drive downstream gene expression changes. To tackle chromatin accessibility, we are performing an assay for transposase-accessible chromatin followed by next-generation sequencing (ATAC-seq), which makes use of a transposase enzyme (which natively shuffles around transposable elements in the genome) to cut DNA into small segments and ligate on adapters for whole-genome sequencing. Because the enzyme preferentially cuts in euchromatic (open chromatin) areas, the regions sequenced represent actively used gene regulatory elements. We are also performing chromatin immunoprecipitation followed by
sequencing (ChIP-seq) to histone modifications, such as acetylated H3K27, which defines active enhancer elements in the genome, as well as methylated H3K27, which denotes repressed chromatin. These experiments will reveal the chromatin states that drive sexual differentiation of the mammalian brain.

The Molecular Basis of Social Attachment

This work was done in collaboration with M. Schatz (Cold Spring Harbor Laboratory).

Deficits in social cognition are a hallmark of many psychiatric disorders; however, it has been difficult to explore the molecular underpinnings of such deficits in the lab as the conventional lab mouse does not display social attachments outside of the mother–pup bond. The prairie vole (*Microtus ochrogaster*) is a mouse-sized rodent that forms strong social bonds, both between adult partners and between parent and offspring. On mating, voles form lifelong pair bonds and thereafter prefer to socially interact with their partner rather than novel mates, spending the majority of their time “huddling” in close physical proximity. Bonded males defend their mates from other voles, attacking both intruder males and unfamiliar females, and parents of both sexes are equally attentive to their pups. Prairie voles may be contrasted with the meadow vole (*Microtus pennsylvanicus*), a closely related species that is solitary in the wild, does not form pair bonds, and has uniparental care of offspring. In collaboration with the lab of Michael Schatz, we are comparing the genomes and neural transcriptomes of these species with those of primates and mice to identify the molecular substrates that mediate social attachment.

The first questions we are asking with this system are how does forming a pair bond alter gene expression in the brain and are these changes the same in the two sexes? We are performing RNA-sequencing from age-matched pair-bonded and same-sex group-housed males and females in the lateral septum, a brain region known to be involved in pair-bonding and partner recognition. We will then assess the brain-wide expression pattern of candidate genes from this screen, to begin to understand how pair-bond formation permanently alters the repertoire of social behaviors displayed by *M. ochrogaster*. To aid this project, we have recently completed de novo sequencing and assembly of the genome of the nonsocial vole *M. pennsylvanicus*. This resource permits comparative analysis to identify genomic regions associated with social behavior that are present in *M. ochrogaster* and primates, but not in *M. pennsylvanicus*, lab mice, and rats. Transcriptomic analysis in the developing brain of multiple rodent species will reveal the gene programs that direct the development of the neural circuits involved in pair-bonding. Our long-term goal is to describe the regulatory genomic landscape that has evolved to drive the formation of social attachment neural circuitry and to leverage this information to better understand human genetic variants implicated in psychiatric disorders.
NEURAL CODING AND MEMORY FORMATION IN THE DROSOPHILA OLFACTORY SYSTEM

G. Turner  M. Brill  T. Hige  M. Modi

The brain has a tremendous capacity to form different memories. In normal circumstances, these memories are highly accurate for particular stimuli and situations. How do patterns of neural activity in the brain convey this specificity? We address this question—the accuracy of memory—by studying the formation of olfactory memories in Drosophila.

We focus on a brain area known as the mushroom body (MB). This area is essential for learning Pavlovian associations between odors and reward or punishment. Unlike earlier layers of the olfactory circuit, MB neurons show highly odor-specific responses, and activity patterns in the MB are relatively sparse. This specificity is thought to underlie the accuracy of memory, as modifying the synapses of highly odor-specific neurons would lead to relatively precise memories. Sparse representations by highly stimulus-specific neurons are a general feature of brain areas involved in learning and memory, including hippocampus and cerebellum.

Using the simplicity and genetic manipulability of Drosophila, our goal is to understand several fundamental properties of sparse representations. What mechanisms give rise to the stimulus specificity? What exactly is meaningful about the activity patterns? Does the precise timing of activity matter, or is it simply which cells respond? How are sparse activity patterns modified by learning?

MB Output Neurons and Flexible 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 laboratory at Janelia Farm, we have functionally characterized odor coding in the complete set of 34 MB output neurons (MBONs). Our main findings are as follows.

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 that 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 experimental 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. We found that MBONs do a poor job of representing odor identity, especially in comparison to the upstream neurons that comprise 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 comprised of odors that are repellent to flies, whereas another group contains food-based odors. This is an exciting result because few people have considered how different sensory inputs can be categorized into behaviorally meaningful classes. We may be seeing olfactory information shaped into behaviorally relevant neuronal signals at this layer—finding the connection between sensory input and motor output.

These two results indicate that MBON odor responses are highly plastic, and the MBON response pattern signals the positive or negative quality of an odor. Together these findings suggest that the MB
Research
represents odors categorically, and experience could potentially shift a neutral odor into either a positive or negative category, depending on the fly’s past experience with that odor.

Synaptic Plasticity in the Olfactory Circuit
T. Hige

Despite the long history of studying learning in *Drosophila*, nobody has actually shown where and how synaptic plasticity occurs. To look for plasticity, we used an optogenetic training regime that allowed us to examine synaptic strength during memory formation. We trained flies to avoid an odor by pairing that odor with optogenetic stimulation of particular dopaminergic neurons. The dopaminergic neurons essentially provide a punishment signal to the animal, substituting for the electric shocks delivered to the animal’s feet in the normal training. Pairing a particular odor with dopaminergic stimulation trains flies to form an aversive association with that particular smell.

These dopaminergic neurons project to the area of the MB where the MBONs are located, and where we identified a potential downstream target to undergo plasticity. Intracellular recordings from this candidate showed a long-term decrease in synaptic strength of the inputs to this neuron. The characteristics of this synaptic depression mirror the learning behavior itself: (1) induction of plasticity requires that the odor “predicts” the arrival of the artificial punishment and (2) the depression is long-term, lasting at least an hour, which is as long as we can reliably measure its time course.

There is an interesting anatomical organization in this region of the brain—the MB output region is organized into a series of compartments. Each of these compartments is innervated by a particular MBON and a corresponding dopaminergic neuron(s). We showed that this modular anatomy reflects an interesting corresponding functional modularity. We found that plasticity can be very easily induced in some compartments, requiring only brief odor exposure and a few spikes from dopaminergic neurons. However, in another compartment, induction required a longer odor exposure with more dopaminergic stimulation. These compartmental differences appear to be related to short-term versus long-term memory formation. Aside from establishing the basic rules governing how synaptic strength is changed in this system, these results open the way to studying the role of various learning and memory-related genes in the induction and maintenance of synaptic plasticity.

Dopamine Controls the Signal-to-Noise Ratio of Signal Transmission through the MB
M. Modi

Neuromodulators, such as dopamine and octopamine, are essential for learning, but how they act and what their precise role is during the learning process have not been established. We examined the effects of these neuromodulators on activity in the MB. Specifically, we tested the effects of increasing steady-state levels of dopamine by directly applying it to the brain. 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.

Additionally, we showed that changing baseline levels of dopamine diminishes odor responses in the neurons that are downstream from the MB. Together, these results suggest a model in which 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.

Is the Timing of Spikes Important for the Neural Code in the Olfactory System?
M. Brill

Neuronal signals propagate from one cell to the next via electrical impulses, or spikes, that evoke neurotransmitter release. These trains of spikes can be considered like the ones and zeros of the binary code of
a computer. Spike trains have many different features that could potentially carry information—the rate of spikes, the timing of the onset, and the relative timing of firing across neurons. Although spike rate is widely recognized as important, other features of those spike trains more related to spike timing could also be important signaling factors. Understanding what features of these spike trains convey information is a fundamental aspect of understanding the brain. It is a little like finding the key to a computer’s binary code.

We have begun investigating this fundamental question by constructing artificial spike trains with these different features (rate vs. latency) present in different degrees. Using optogenetic techniques, we can control the activity patterns at an early layer of the olfactory system. By then monitoring downstream layers of the circuit using in vivo imaging techniques, we can assess what features of those artificial spike trains are effectively transmitted to those downstream neurons (i.e., what features of the code are relevant). The project is at an early stage, and we are currently focusing on the importance of the onset of spiking activity. If inhibition is slow and takes time to develop, early-onset spikes could potentially be more effective at driving downstream circuitry than those arriving late. Combining this optogenetic control of spike trains with measures of the impact on behavior could reveal which spike train features are important for the perception of the animal, getting to the core features of the neural code in this system.

PUBLICATIONS
CIRCUITRY UNDERLYING CORTICAL PROCESSING AND DECISION-MAKING

A. Zador  B. Burbach  D. Gizatullina  F. Marbach  C. Stoneking  A. Zhang
F. Carnevale  J. Kebschull  I. Peikon  V. Vasily
X. Chen  U. Livneh  A. Reid  Q. Xiong

Our 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. In addition, we are developing a method that will allow us to use high-throughput DNA sequencing to determine the wiring diagram of the brain at single-neuron resolution rapidly and efficiently, at low cost.

Optogenetic Manipulation of Striatal Projection Neurons during Auditory Discrimination
F. Carnevale

We have previously shown that optogenetic activation and inactivation of corticostriatal neurons or axons can bias behavioral choices during an auditory discrimination task (Znamenskiy and Zador, *Nature* 497: 482 [2013]). Others have shown that optogenetic manipulation of direct and indirect striatal neurons during other behaviors facilitates and inhibits movement (Tai et al., *Nat Neurosci* 15: 1281 [2012]). We are therefore testing whether manipulating neuronal activity in auditory striatum, a region of striatum receiving information from auditory cortex, can bias behavioral choices during auditory discrimination. Previous work in the lab has shown that corticostriatal plasticity correlates with learning the sensorimotor association (Xiong et al. 2015). We therefore hypothesize that, because striatal neurons are downstream from this site of plasticity, the effect on choice of manipulating their activity will be independent of the trained association. These experiments will help test the general hypothesis that the corticostriatal pathway provides a mechanism for the formation of arbitrary sensorimotor transformations.

High-Throughput Neuronal Identification and Characterization at Single-Cell Resolution in Visual Cortex
X. Chen

Current technologies allow probing multiple aspects of neuronal properties at single-cell resolution. Registering these multiple properties to the same neurons within a population, however, is difficult. To overcome this problem, we are developing a technique to combine multiplexed analysis of projections by sequencing (MAPseq), in situ sequencing, and calcium imaging to read out neuronal projections, cell types, and activities of a neuronal population at single-cell resolution. We then combine all three types of information using the locations of the neurons. This technique would allow us to gain insights into how different neuronal properties correlate with each other at the cellular level.

Role of Corticostriatal Plasticity in Auditory Decisions
Q. Xiong

Corticostriatal plasticity plays a key role in reinforcement learning, but how associations between stimuli and motor responses are established remains unclear. Recent work from our group showed a causal role of corticostriatal neurons in driving choices during an auditory discrimination task, 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
responses. Because all sensory cortical areas send projections to the striatum, our findings suggest a general mechanism for the formation of arbitrary sensorimotor transformations.

Cortical Representations of Self-Generated Sounds in Mice
U. Livneh

Auditory-guided behaviors, such as speaking and playing music, constitute a hallmark of human behavior. These behaviors require us to execute a sequence of motor movements with a remarkable precision and speed. To do so, our nervous system ought to monitor the acoustic signals that are generated by our own movements and to generate a feedback signal that can be used, in turn, by the motor system to correct and adjust our movement. Presently, ample evidence suggests that the motor system is directly involved in modulating auditory cortex responses to self-generated sounds. However, the neural mechanisms that underlie this process remained poorly understood. To address this question, we have recently developed a novel behavioral task that enables us to investigate the neural mechanisms that underlie the representation of self-produced sound in head-fixed mice. In the task, mice are trained to control a computer-controlled “musical instrument”—a mousephone—that transforms their movement into real-time auditory feedback. Mice readily learn to control the device and use the auditory feedback to guide their movement. Using this task, we have shown that a large fraction of neurons in the auditory cortex selectively respond to auditory signals that are produced by the mouse’s own movements. Currently, we are investigating the neuronal mechanisms that underlie this sensory selectivity using two-photon calcium imaging and electrophysiological recordings. These investigations are expected to allow us to obtain insight into the neural mechanisms underlying these behaviors in mice and may ultimately provide insight into the mechanisms underlying a range of human auditory behaviors.

Corticostriatal Activity and Its Role in an Auditory Discrimination Task
F. Marbach

We are interested in the circuits underlying the representations of sounds and how these representations are used for auditory-guided behavior. In particular, we are interested in corticostriatal cells, a population of neurons that projects from the auditory cortex to the striatum, a brain region important for action selection. Previous work in our lab has shown that the activity of corticostriatal cells can influence the decisions made by rats in an auditory task. We therefore hypothesize that these cells convey information about the auditory stimulus used to select appropriate actions by the striatum. To study the activity of corticostriatal cells and contrast it with that of other neural populations projecting out of auditory cortex, we use two-photon calcium imaging in head-restrained mice performing an auditory discrimination task. This work will help us understand how information from the auditory environment that is ultimately used to select a desired action is distributed across different brain areas.

Sequencing the Connectome
I. Peikon, D. Gizatullina, J. Kebschull, Y. Sun, V. Vagin

We are developing an entirely novel approach of mapping neural connectivity, which harnesses the high-throughput power of next-generation sequencing. We have engineered a molecular tool set to barcode individual neuronal connections (BOINC) for readout via DNA sequencing.

The set includes pre- and postsynaptic proteins that bind with distinct RNA barcodes and a chemical cross-linker that covalently links these proteins across a synaptic cleft. Isolation of the cross-linked proteins with synaptic RNA barcodes and their conversion into joint DNA products bring neural connectivity onto DNA sequencing.

We have shown BOINC allows the isolation of the cross-linked complex containing pre- and postsynaptic proteins and RNA barcodes from synaptic cleft. Now we are optimizing an enrichment method of cross-linked complexes with both pre- and postsynaptic RNA barcodes. To convert them into joint DNA products that reflect individual synaptic connections, we have been developing a single-molecule joining method.

Sequencing the Projectome
J. Kebschull, L. Huang, A. Reid, I. Peikon

Understanding the brain requires knowledge of its anatomy. Recently, much progress has been made in
mapping out “mesoscale” (area-to-area) connectivity in the mouse brain. However, most current approaches rely solely on bulk mapping of projections and cannot resolve the projection patterns of single neurons; techniques with the resolution needed to resolve single-neuron connectivity suffer from very low throughput.

We have developed a high-throughput, multiplexable method to determine long-range projections in the mouse brain at single-cell resolution, called MAPseq. To do so, we are leveraging the vast combinatorial space of nucleic acid barcodes to uniquely label individual neurons. We then use the power of modern high-throughput sequencing technology to reliably, quickly, and inexpensively read out these labels at projection target sites.

As a proof of principle, we applied MAPseq to locus coeruleus (LC), a small noradrenergic nucleus that projects throughout the brain. In contrast to previous bulk labeling studies that report diffuse and nonspecific projections from the LC, our single-neuron resolution analysis reveals that most individual LC neurons have preferred cortical targets. Our findings suggest that LC may exert differential control over cortical areas as opposed to the broadcasting model for LC supported by lower-resolution bulk projection data.

MAPseq is not limited to tracing projections from one area, but it scales to map the projections of neurons from many brain areas in a single animal. This both minimizes the labor and cost of brain-wide projection mapping and eliminates the need to map data from several animals to an average reference frame. Currently, we are using MAPseq to determine the entire corticocortical mesoscale connectome at single-cell resolution in a single experiment.

Proximity Ligation Assay for Synapse Detection
J. Kebschull

We are developing a novel method to label synapses between two genetically identified neural populations based on the proximity ligation assay (PLA). PLA uses antibodies to detect the close (<40 nm) apposition of two proteins. By targeting a pre- and postsynaptic protein, we can identify synapses with high reliability.

Role of Corticostriatal Plasticity in Stimulus-Motor Association
S. Ghosh

Previous work from our lab has shown that corticostriatal projections play a causal role in decision-making in a complex auditory discrimination task (Znamenskiy and Zador, *Nature* 497: 482 [2013]; Xiong et al. 2015). Moreover, learning of this discriminatory task results in a specific pattern of plasticity in this pathway, which is determined by the frequency tuning of the neurons and the corresponding motor movement. Nevertheless, how this pattern of plasticity overlays onto the complex striatal circuitry remains unknown. We are therefore testing the hypothesis that differential patterns of plasticity in specific subtypes of corticostriatal synapses underlie the ability to associate a particular auditory cue with a given motor action in the “tone cloud” task.

Imaging Striatal Activity in Mice Making Auditory Decisions
C. Stoneking

Although decision-making has been extensively studied, the basic mechanisms by which animals learn to make decisions remain unclear. Recently, our group found that the activity of projections from cortex to striatum determines the decisions that rodents make on an auditory discrimination task. Subsequent work showed that the strength of these projections changes during learning, in a manner that reflects the association learned. Based on these findings, we will test the hypothesis that learning the task is implemented by changes in striatal activity. To do so, we will track striatal activity as animals learn the task, using deep imaging with an implanted relay lens and miniaturized head-mounted microscope.

Role of Corticostriatal Pathway in Sensory Decision-Making
A. Zhang

Neurons that project from auditory cortex to striatum play a causal role in guiding performance in an auditory decision-making task (Znamenskiy and Zador, *Nature* 497: 482 [2013]; Xiong et al. 2015). We will
now use optogenetic manipulations to test whether the corresponding projection neurons from visual cortex play a similar role in an analogous visual task. This will allow us to understand whether the circuit mechanism underlying auditory decision-making is shared across sensory modalities.

**PUBLICATIONS**


NEURAL BASIS OF LEARNING AND MEMORY IN DROSOPHILA

Y. Zhong  J. Beshel  Y-C. Shuai

We combine genetic and functional analyses to study the cellular and molecular bases of learning and memory in *Drosophila*. One of the major approaches we are undertaking is to start our research with genes identified in human disorders with a component of cognitive dysfunctions. Currently, we are working with genes indicated in neurofibromatosis 1, Alzheimer’s disease, and autism. Studies of these genes lead us to gaining new insights into brain functions that would otherwise have been difficult to work with, such as forgetting, spacing effect, and memory retrieval. Another major effort has been devoted to imaging brain activities of living flies, and we hope we will be able to gain insights into sensory coding and memory traces of such coding. Such an effort led us to identify “perceptual coding” neurons, through which we believe that sensory coding is transformed into perceptual coding that directly predicts behaviors. Some of our research is detailed below.

Dissecting Neural Pathways for Forgetting of Olfactory Aversive Memory

Y-C. Shuai

Recent studies have identified molecular pathways driving forgetting and supported the notion that forgetting is a biologically active process. The circuit mechanisms of forgetting, however, remain largely unknown. Here we report two sets of *Drosophila* neurons that account for the rapid forgetting of early olfactory aversive memory. We show that inactivating these neurons inhibits memory decay without altering learning, whereas activating them promotes forgetting. These neurons, including a cluster of dopaminergic neurons (PAM-β’1) and a pair of glutamatergic neurons (MBON-γ4 > γγ1γ2), terminate in distinct subdomains in the mushroom body (MB) and represent parallel neural pathways to regulate forgetting. Interestingly, although the activity of these neurons is required for memory decay over time, it is not required for acute forgetting during reversal learning. Our results thus not only establish the presence of multiple neural pathways for forgetting in *Drosophila* but also suggest the existence of diverse circuit mechanisms of forgetting in different contexts.

Conserved Genetic and Neural Circuits to Modulate Obesity-Linked Behaviors

J. Beshel

Leptin, a typically adipose-derived “satiety hormone,” has a well-established role in weight regulation. Here we describe a functionally conserved model of genetically induced obesity in *Drosophila*, by manipulating the fly Leptin analog Unpaired 1 (upd1). Unexpectedly, cell-type-specific knockdown reveals that upd1 in the brain, not in the adipose tissue, mediates obesity-related traits. Disrupting brain-derived upd1 in flies leads to all the hallmarks of mammalian obesity: increased attraction to food cues, increased food intake, and increased weight. These effects are mediated by domeless receptors on neurons expressing *Drosophila* neuropeptide F, the orexigenic mammalian neuropeptide Y homolog. In vivo two-photon imaging reveals upd1 and domeless inhibit this hedonic signal in fed animals. Manipulations along this central circuit also create hypersensitivity to obesogenic conditions, emphasizing the critical interplay between biological predisposition and environment in overweight and obesity prevalence. We propose adipose- and brain-derived upd/Leptin may regulate different aspects of weight or body size regulation through distinct circuits.

PUBLICATIONS

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-generation profiling and chromatin immunoprecipitation methods that have generated many new hypotheses regarding 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, and 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 do 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, the lab is 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.
Our research aims to identify the ways in which plant growth is controlled, with the ultimate goal of improving crop plant yield. More specifically, we identify genes, signals, and pathways that regulate plant architecture and development. Organisms develop by carefully controlling the passage of information molecules between cells during development. We are interested in discovering the signals that carry this information, finding out how the signals are transmitted, and how they function.

A major focus has been identification of genes that control stem cell maintenance and identity. Examples from the past year include discovery of a new pathway in stem cell control using feedback signals from differentiating cells, a transcription factor that controls the way in which stem cell fate is controlled, and a redox enzyme that controls its activity. We also continue to characterize the role of G proteins in stem cells, identifying candidate upstream receptors, as well as mutations in partner proteins, using CRISPR (clustered regularly interspaced short palindromic repeats) genome editing, which, as in many systems, is revolutionizing the way we do genetics. Our discoveries help explain how signaling from diverse receptors is achieved in plants. We also continue to generate genetic tools for the maize research community by creating a collection of lines that can drive expression of any reporter or experimental gene in any tissue type. Such tools are of great interest to researchers and are being made available to the broader scientific community, enabling experiments never before possible in crop plants.

The Regulation of Meristem Size in Maize

B.I. Je, Q. Wu, F. Xu, J. Fabiano, T. Eck [in collaboration with A. Eveland, Donald Danforth Plant Science Center; 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.

Recently, we have shown that the maize COMPACT PLANT2 (CT2) gene, which encodes the predicted α subunit of a heterotrimeric GTPase (Gα), functions in the CLAVATA pathway to control meristem size through its interaction with FEA2. Genetic data indicate that ct2 and fea2 are in a common pathway, and coimmunoprecipitation shows that the proteins are in the same complex. Interestingly, our Co-IP experiments in Nicotiana benthamiana suggested that scrambling the cytoplasmic tail of the FEA2 receptor did not affect the FEA2–CT2 interaction, suggesting their interaction may require additional mediators. Furthermore, FRET and BiFC data also suggested that FEA2 and CT2 do not directly associate with each other, and other proteins may bridge their interaction. To find the bridging protein(s), we used immunoprecipitation mass spectrometry (IP-mass spec)
to find proteins that can be pulled down by both FEA2 and CT2. We identified an uncharacterized leucine-rich repeat receptor-like kinase (LRR-RLK) that interacted with both FEA2 and CT2, and these results were confirmed by Co-IP experiments using a tobacco transient expression system. Furthermore, BiFC results suggested that the LRR-RLK protein directly associates with CT2, but a kinase domain truncated version did not, suggesting the kinase domain is required for this interaction. This LRR-RLK is preferentially expressed in meristems, and we are now searching for phenotypes, to understand its biological function in meristem development.

The G protein β subunit coordinates with Gα for signal transduction, and there is evidence that this gene controls meristem size in Arabidopsis, so we used CRISPR to knock out the maize Gβ gene, ZmGB1. Surprisingly, we found that zmgb1 null alleles cause cell death and seedlings die at an early stage. We are trying to figure out the reason for this lethality by checking embryo development and immune responses of zmgb1 alleles. Arabidopsis Gβ alleles are viable, and there is evidence for a role in defense responses, so our findings could bring important new insights. In addition, we are using CRISPR to knock out the extra large G proteins (XLGs), which have a Gα homology domain at their carboxyl terminus. There are three XLGs in maize, and we generated different alleles for each of the three proteins using CRISPR. We are crossing the xlgs with ct2 mutants to obtain quadruple mutants to study the genetic relationship between ct2 and xlgs.

To further understand the functions of CT2 in meristem regulation, we screened for natural modifiers taking advantage of maize genetic diversity. We crossed ct2 mutants in B73 to each of the 25 nested association mapping (NAM) parental maize inbred lines. We found the ct2 fasciated ear phenotype was dramatically enhanced in NC350 and HP301 backgrounds and suppressed in CML69. In the enhanced plants, the ear inflorescence meristems were extremely fasciated and showed multiple branches. By using scanning electron microscopy, the enhanced fasciation phenotype was detectable at very early stages of ear development (~2-mm ear length) (Fig. 1). The ct2 suppressors in the CML69 background only suppressed the fasciated ear phenotype, whereas other ct2 phenotypes, such as dwarf, wide, and erect leaves, and dense spikelets were not affected, indicating the suppressor may function specifically in ear inflorescence meristem regulation. We are mapping the enhancer and suppressor loci to better understand the roles of heterotrimeric G proteins in meristem regulation.

Another fasciated ear mutant that we have cloned is fasciated ear 3 (fea3), which was derived from irradiation mutagenesis. fea3 also shows an overproliferation of the inflorescence meristems, leading to enlarged or fasciated ears and thick tassels. Double mutants of fea3 with td1 or fea2 have additive and synergistic phenotypes, suggesting that they act in independent pathways that converge on the same downstream target to control meristem size. FE3 encodes a predicted leucine-rich repeat receptor-like protein related to FEA2. In situ hybridization and imaging of red fluorescent protein (RFP)-tagged transgenic plants show that FEA3 is expressed in the organizing center of the shoot apical meristem (SAM) and in leaf primordia, as well as in the root apical meristem. Remarkably, expression of maize WUSCHEL, a marker for the stem cell niche-organizing cells, spreads downward in fea3 mutants, which is strikingly different from its response in the known CLAVATA stem cell mutants.

FEA3 is localized in the plasma membrane, and to ask if it 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, ZmFCP1 (FLORAL ORGAN NUMBER2-like CLE protein 1), compared with FEA2. ZmFCP1 is expressed in leaf primordia, suggesting that it serves as a signal from differentiating cells to repress meristem growth via the
FEA3 receptor. Our results suggest that the FEA3 receptor functions in a new pathway for stem cell control that is spatially distinct from the known CLV receptors and uses a different peptide signal. This signaling system appears to be universal, as we have also found orthologs in Arabidopsis with similar properties. We are also using CRISPR technology to knock out four close homologs of FEA3 in maize to investigate their potential function in meristem development.

We found that weak alleles of fasciated ear mutants could improve maize yield traits, such as kernel row number (KRN), by increasing meristem size and number of primordia while maintaining structural integrity of the meristem. To ask if fea3 weak alleles have similar effects, we backcrossed fea3 ethyl methanesulfonate (EMS)-induced alleles developed in our noncomplementation screen to W22 or B73 inbred lines, and generated F1 hybrids. As expected, the B73/W22 wild-type hybrids had significantly enhanced yields compared with the inbreds because of heterosis. We found that fea3-2 and fea3-3 weak allele hybrids further enhanced ear length, KRN, kernel number per ear, and ear weight (Fig. 2). The weak alleles were mildly fasciated, but did not display the stunted ear growth that is normally associated with strongly fasciated mutants. These results are particularly exciting because in our previous studies of weak fea2 alleles, we found an increase in KRN, but no overall increase in ear weight because of a compensatory reduction in kernel size. Therefore, the newly identified FEA3 signaling pathway could be used to develop new alleles for crop improvement.

Aberrant phyllotaxy 2 (Abph2) is a gain-of-function mutant in maize with enlarged shoot meristems and a decussate phyllotactic pattern. Previous studies in our lab showed that this mutation is caused by transposition of a glutaredoxin gene, MALE STERILE CONVERTED ANther1 (MSCA1), resulting in altered expression pattern of MSCA1 in Abphyl2. Consistently, msc1 loss-of-function mutants have reduced meristem size. The growth defect of meristems in the Abphyl2 and msc1 mutants suggests a potential novel function of glutaredoxins in meristem development. Interestingly, MSCA1 interacts with a TGA transcription factor, FASCIATED EAR4, and these proteins overlap in expression in the nucleus of some cells of the SAM and inflorescence meristem. Genetic analysis showed that fea4 is epistatic to msc1 with respect to SAM size, thus supporting the idea that they interact in a pathway. To investigate how the activity of FEA4 is regulated by MSCA1, we crossed FEA4-YFP and FEA4-RFP transgenic plants with msc1 mutants. Experiments are in process to investigate how MSCA1 regulates the function of FEA4 and other putative targets through posttranscriptional modification. CRISPR technology has also been used to successfully knock out two MSCA1 homologs to further investigate the function of glutaredoxin genes in meristem development.

Along with these advances, we are mapping additional fasciated ear mutants from EMS mutagenesis screens. We have determined candidate map positions for several of these mutants and are proceeding with fine mapping and molecular cloning. For one of the mutant fea148, the mutation maps between 221 Mbp and 236 Mbp on chromosome 4. For another mutant fea184, the mutation maps between 126 Mbp and 134 Mbp on chromosome 3. For each mutant, multiple homozygous SNP mutations were identified within the mapping region by using whole-genomic sequencing approaches. To identify the causal mutation, additional alleles of Mu transposon insertion lines for the
candidate genes form the maize stock center and are being introgressed for further phenotype characterization. Meanwhile, larger mapping populations are being used to further narrow down the map locations.

Control of Shoot Branching and Determinacy
H. Claeys, E. Demesa-Arevalo, H. Hong, T. Skopelitis [in collaboration with A. Eveland, Donald Danforth Plant Science Center; M. Komatsu and H. Sakai, DuPont Crop Genetics; B. Yang, Iowa State University]

The RAMOSA (RA) genes in maize function to impose determinacy on axillary meristem growth; consequently, ra loss-of-function mutants (ra1, ra2, and ra3) have more highly branched inflorescences. RA3 encodes a trehalose phosphate phosphatase, which catalyzes the conversion of trehalose-6-phosphate (T6P) to trehalose. T6P is an important regulatory metabolite that connects sucrose levels, and thus the sugar status, to plant growth and development, but its mode of action is still unclear. RA3 is expressed in a localized domain at the base of axillary inflorescence meristems and localizes to distinct puncta in both nuclear and cytoplasmic compartments, suggesting that its effect on development may not be 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 because it affects the expression of specific genes.

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 enhancers 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 in the upper part of the ear. ra3 mutants in the Mo17 background were also mutagenized: ra3 is suppressed in Mo17, so the presence of branches also indicates an enhancer mutation. Combining both screens, roughly 20 mutants have been isolated so far. Mapping of a subset of these mutants is currently under way. So far, two independent mutant alleles of REL3 (RAMOSA ENHANCER LOCUS 3), an RA3 paralog, were identified. Both contain amino acid substitutions, and we are currently trying to understand how these mutations affect protein activity. Another mutant was mapped to a gene encoding an RNA-binding protein that is known to play a role in inflorescence development, and we are also focusing on understanding this genetic interaction.

In a parallel approach to understand how RA3 functions, we are screening for interacting proteins. To this end, a yeast two-hybrid screen was performed, and a number of predominantly nuclear proteins were found to interact with RA3, which fits with its partially nuclear localization. In parallel, ear primordia from plants expressing an HA-tagged RA3 protein were used for immunoprecipitation followed by mass spectrometry (IP-MS), revealing a number of potential in vivo interactors. The biological roles of a number of these interactors are currently being studied using insertional mutants, and mutant alleles generated using CRISPR–Cas9. In this regard, we are currently analyzing the progeny of CRISPR alleles in two gene families of RA3 interactors: the RNA recognition motif (RRM) Zea mays SCAFFOLD ATTACHMENT FACTOR B (ZmSAFB) and ZmSAFB-like and the vascular plant one-zinc-finger (ZmVOZ) transcription factors, to analyze their genetic interactions with ra3 (Fig. 3).

Finally, we are combining a number of approaches to directly understand the contribution of the enzymatic function of RA3 to its biological function. We are complementing the ra3 mutant with a catalytically dead mutant version of RA3 generated by bacterial artificial chromosome (BAC) recombineering. We are also using CRISPR–Cas9 to generate mutants in TREHALOSE PHOSPHATE SYNTHASES (TPSs), which catalyze the preceding step to RA3 in the trehalose metabolic pathway. If accumulation of T6P is causing the ra3 phenotype, then combining ra3 with tps mutants should alleviate the phenotype, giving important insights into its mechanism.

Natural Variation in Inflorescence Architecture
H. Claeys, J. Fabiano, T. Eck, K. Vera, M. Rutigliano [in collaboration with S.L. Vi, The Agriculture Genetics Institute, Hanoi]

Maize inflorescence architecture has 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, but 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 or suppress the phenotypes of different mutants. We focused on the 25 NAM founder inbreds, because they were selected to capture the diversity of maize germplasm, and because of the genetic tools available for these lines. We have crossed these 25 inbreds to our collection of mutants (in a B73 background) and screened the F2 for plants with suppressed or enhanced phenotypes compared with the mutant in the B73 background.

We identified a suppressor of *ramosa3* (*ra3*) from the Mo17 inbred and an enhancer coming from the Ki11 inbred, as well as an enhancer of *fea2* coming from NC350. Segregation ratios suggest one or two loci in each case, suggesting they will be genetically tractable. 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 (QTLs) rather than single-gene effects, we crossed the mutants to the corresponding NAM-founder/B73 recombinant inbred lines (RILs), to identify and map potential modifying QTLs. Using this approach, we successfully mapped the *fea2* enhancer from NC350 to a region that colocalizes with a previously identified KRN QTL. This fits with our lab’s recent finding that hypomorphic alleles of *fea2* lead to increased KRN, linking fasciation and KRN. We are currently validating a candidate gene within the identified region. Additionally, we have candidate map regions for two *ra3* enhancers and a suppressor from Ki11, which we are also in the process of fine mapping.

The Effects of Drought on Early Inflorescence Development

H. Claey [in collaboration with H. Cline and B. Meeley, DuPont Crop Genetics]

Drought stress is one of the major environmental factors limiting maize yield. Some progress has been made in studying how drought affects grain filling during the later stages of reproductive development, but almost nothing is known about the molecular mechanisms through which drought has an effect on early inflorescence development, in which the number of potential grains is being determined. It has been found that yield is significantly affected when maize is subjected to drought at this stage. Therefore, we set out to address this question as part of a larger collaboration with DuPont-Pioneer and the Eveland lab at the Danforth Center, the Vollbrecht lab at Iowa State University, the Dinneny lab at the Carnegie Institution, and the Hake lab at University of California at Berkeley.

Plants were grown in automated greenhouses at a Pioneer facility in Iowa, and water was withheld from...
half of the plants during early ear development. We harvested small ears (<1 cm) from these plants before the onset of the drought treatment and at two time points following drought onset. Ear tips, containing the inflorescence meristem, were dissected, and their transcriptomes were profiled. This revealed a large transcriptional reprogramming of inflorescence meristems in response to drought, affecting many known developmental regulators. We identified the gibberellin and T6P pathways as potential mediators of this response and are currently following up on this. Additionally, we are performing a second large transcriptome experiment to identify early signaling events after drought with even greater developmental resolution.

Generation of a pOp-LhG4 Transactivation System for High-Throughput Protein Localization and Functional Studies in Maize

E. Demesa-Arevalo, Q. Wu, B. Il Je, T. Skopelitis
[in collaboration with A. Chan, J. Craig Venter Institute; A. Sylvester, University of Wyoming]

Despite the growing abundance of resources available for the study of various model organisms, the classical approach of characterizing a single gene mutant still provides invaluable information regarding gene function. Visual information in regard to time and location of gene expression and the ability to study gene products at the protein level complement the genetic approaches to study the biological role of a gene. We previously generated transgenic lines tagging various genes with fluorescent markers using their endogenous promoters. Using our experience in promoter analysis, we are currently establishing tools for complex analysis at cellular, tissue, or organ level. We have developed a pOp-LhG4 transactivation system in maize that allows us to express proteins in specific tissues and/or transient stages during development. The pOp-LhG4 system includes (1) a transcription activator, LhG4, which is a fusion between a high-affinity DNA-binding mutant of lac repressor, LacPhis17, and transcription-activation-domain-II of GAL4 from Saccharomyces cerevisiae, and (2) a chimeric promoter, pOp, that consists of lac operators cloned upstream of a minimal CaMV promoter, not activated in the reporter lines until crossed with the LhG4 activator line. We have already generated many LhG4 drivers, as well as seven pOp responder lines, including Zea mays FON2-LIKE CLE PROTEIN1 (pOp::FCP1) and the FLOWERING LOCUS T-like Zea mays CENTRORADIALIS 8 (pOp::ZCN8) and MALE STERILE CONVERTED ANther 1 (pOp::MSCA1). We confirmed the reliability of our system by crossing pOp::ZCN8 plants with a constitutive promoter line, pEF1A::LhG4 and 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. Now, we are analyzing additional tissue-specific LhG4 driver lines expressing pOp::MSCA1, under a constitutive promoter line, and pYABBY14-LhG4, a driver line expressed in young leaf primordia, but not in the SAM. Using this system, we are trying to understand when and where the expression of MSCA1 can drive alterations in plant architecture because misexpression of this gene is the cause of Aberrant phyllotaxy 2 (Abph2) phenotypes. Under the pYABBY14 promoter pOp::MSCA1 is able to phenocopy the embryo shape of Abph2, but not the decussate phyllotaxy (Fig. 4, upper panels). Currently, approximately 20 LhG4 driver constructs have been generated, and our goal is to produce a comprehensive array of cell- and tissue-specific lines, which we will use to (1) isolate specific cell types by fluorescence-assisted cell sorting (FACS) for transcriptional profiling (Fig. 4, lower panels), and (2) drive the misexpression of developmentally regulated genes to better understand the function of stem cell niches during 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.

Role of SHOOTMERISTEMLESS Trafficking in the SAM

R. Balkunde

SHOOTMERISTEMLESS (STM) encodes a homeodomain transcription factor and is required for the maintenance of pluripotent stem cells in the shoot. STM is expressed throughout the meristem, and is down-regulated in developing organ primordia. STM
protein moves between cells, but the significance of this is not well understood. A common approach to prevent cell-to-cell movement is to add nuclear localization sequences (NLSs) to sequester the protein in the nucleus. We made an STM native expression construct with YFP fused at the amino terminus, and a second construct that also contained 2xNLS sequences at the amino terminus, to promote nuclear import. We found that the native pSTM::YFP~STM construct fully rescued \textit{stm} phenotypes; however, the pSTM::2xNLS~YFP~STM construct did not. The 2xNLS~YFP~STM plants had axillary shoots in the axils of the cotyledons and smaller SAMs with fewer YFP~STM-expressing cells. We interpret the results as evidence that STM trafficking is important for proper SAM development. For example, having a smaller or “less potent” SAM could lead to axillary bud formation in the axils of cotyledons by a loss of apical dominance. This phenotype also suggested a possible defect in defining a correct organ boundary. It is known that STM and organ boundary genes \textit{CUP-SHAPED COTYLEDON1/2} (\textit{CUC1/2}) reinforce each other. Therefore, we are checking the expression of boundary genes. Subtle differences in the localization of STM and 2xNLS-STM at the organ boundary region may influence the levels of boundary gene expression, which may explain the developmental differences between the lines expressing STM and lines expressing 2xNLS-STM.

**PUBLICATIONS**


*In Press*


Research in our laboratory seeks to reveal, understand, and manipulate the genetic and molecular networks that control when, where, and how flowers are produced on plants. Flowers form on inflorescences, reproductive branches that originate from meristems, which are small groups of stem cells located at the growing tips of all shoots. The number of inflorescences produced on a plant, as well as how many flowers form on each inflorescence, can vary dramatically both within and among species. At the center of this diversity lie two critical processes of meristem growth and regulation: (1) meristem maturation, during which stem cell populations transition from a vegetative to a reproductive state, and (2) meristem maintenance, a process that controls stem cell proliferation and meristem size. We are taking advantage of natural and mutant variation in inflorescence production and architecture in tomato and related Solanaceae species to understand how differences in meristem maturation and size determine the remarkable diversity in flower production and reproductive success observed in nature and agriculture. We are using tomato as a foundation to address these questions because meristems are easily accessed for morphological and molecular analyses, and genetic perturbations have provided a molecular framework for how meristem maturation and size are regulated. Importantly, the Solanaceae family includes major crop and ornamental plants, as well as many edible wild species, making our discoveries directly relevant to improving agriculture.

A Cascade of Arabinosyltransferases Controls Shoot Meristem Size in Tomato
C. Xu, K. Liberatore, S. Thomain, C. MacAlister, C. Brooks

We discovered a new mechanism controlling meristem size that is critical for crop productivity. Two decades of research in Arabidopsis have established that meristem size is controlled through the classical CLAVATA (CLV) pathway. At the heart of this pathway is CLV3, a signaling peptide modified with sugars that binds to the receptor CLV1. However, despite hundreds of studies on the CLV pathway over the last 20 years, the pathway responsible for modifying CLV3, and its significance for meristem size, has been a mystery.

By studying novel “fasciation” mutants of tomato with branched inflorescences that produce flowers and fruits with more organs due to enlarged meristems, we identified the homolog of CLV1. Surprisingly, a much more extreme mutant, fasciated inflorescence (fin), was defective in a hydroxyproline O-arabinosyltransferase (HPAT), an enzyme that adds the first arabinose sugar to arabinose chains on diverse plant proteins (Fig. 1). Using CRISPR–Cas9, we created mutations in arabinosyltransferases predicted to elongate arabinose chains, and these plants were also fasciated because of enlarged meristems, indicating CLV3 requires complete arabinose chains to fully activate CLV signaling and maintain meristem size. Notably, we observed that mutants in the third arabinosyltransferase were the least fasciated, suggesting CLV3 activity is directly proportional to arabinose chain length. These findings exposed arabinosyltransferase genes as critical new components of the CLV pathway, revealing an unexpected and exciting new layer of complexity in the control of stem cell proliferation and meristem size during plant development.

In a related project, we discovered the genetic origins of extremely large tomatoes. Our collection of arabinosyltransferase and CLV pathway mutants was highly reminiscent of naturally occurring fasciation responsible for increased fruit size during tomato domestication. We therefore revisited a classical mutant,
Research

fasciated (fas), which was responsible for the evolution of extreme fruit size and was previously shown to result from a mutation in a YABBY transcription factor gene. However, a deeper dissection of the fas locus in collaboration with E. van der Knaap (Ohio University) revealed a large inversion with a breakpoint just upstream of CLV3. Through a series of molecular and transgenic experiments, we discovered that fas is caused by a regulatory change in CLV3. Thus, modification of CLV3 and CLV signaling was a major target of domestication and is responsible for all modern large-fruited “beefsteak” tomato varieties.

Figure 1. The fab and fin mutants develop branched inflorescences with fasciated flowers as a result of enlarged meristems. (A) An unbranched inflorescence typical of a wild-type (WT) plant and a typical WT flower (inset). (B,C) Primary inflorescences from fab (B) and fin (C) showing branching and fasciated flowers (insets). (D–F) WT fruits produce two locules (D), whereas the fruits of fab (E) and fin (F) develop extra locules. Arrowheads, locules. (G–I) Stereoscope images of the primary meristem from WT (G), fab (H), and fin (I) at the transition meristem (TM) stage before formation of the first flower. Dashed lines mark the width and height.

HPAT Mutants Oppositely Alter Tip Growth in Arabidopsis thaliana and Physcomitrella patens

C. MacAlister, S. Thomain

In the last several years, there have been significant advances and new interest in understanding the function of carbohydrate modifications on plant proteins, particularly hydroxyproline O-arabinosylation. Arabinose chains are initiated by HPATs, the first three of which were identified in Arabidopsis only a few years ago. As mentioned above, we found that mutations in
a tomato HPAT homolog (FIN) cause enlarged shoot meristems as a result of loss of arabinosylation on CLV3. Other reported hpat mutants include a supernodulating mutant in Medicago, and Arabidopsis hpat1 hpat2 double mutants, which display pleiotropic defects, but no change in meristem size. However, a redundant requirement for HPAT1 and -3 for male gamete transmission has prevented an analysis of hpat1,-3 double or hpat1,-2,-3 triple mutant plants. Moreover, deep conservation of the HPAT family suggests critical functions in all plants; yet, the roles of HPATs in basal plants are unknown.

We performed a comparative study of HPAT function in Arabidopsis and the model moss Physcomitrella patens to identify common and lineage-specific functions of hydroxyproline O-arabinosylation. Surprisingly, we found that meristem size is unaffected in either double or complete loss-of-function triple hpat mutant plants in Arabidopsis, suggesting that the prominence of HPAT’s roles in stem cell control varies among species. Instead, the major phenotype we observed in triple hpat mutants was loss of male fertility because of a defect in pollen tube elongation. In parallel, we knocked out the two genes encoding HPATs in P. patens and found that these moss mutants have a defect in filament tip growth (Fig. 2). Interestingly, whereas Arabidopsis hpat pollen tubes are shorter than wild-type, Physcomitrella hpat filaments are longer and faster growing than normal. Using growth assays and global expression profiling, we traced the moss hpat phenotype to a likely defect in cell wall formation.

This comparative study revealed a common function for HPATs in gametophytic tip growth of two important species: Arabidopsis and Physcomitrella. The results highlight the conservation of HPAT functions across species and the importance of cell wall modification in pollen tube growth. Further studies are needed to understand the specific roles of HPATs in these processes.
A critical determinant of crop productivity is plant architecture. For example, tomato production was revolutionized a century ago with the discovery of the self-pruning (sp) mutant, which transformed indeterminate plants into a compact determinate form that enabled both synchronous ripening and mechanical harvesting. The SP gene encodes a repressor of flowering in the CETS protein family, which includes the universal flowering hormone florigen (in tomato, encoded by SINGLE FLOWER TRUSS, SFT). In many crops, selection for mutations in CETS family members during domestication led to a modified balance of flowering signals, resulting in improved shoot architecture, flower production, and productivity.

In prior work, we discovered a genetic toolkit of chemically induced mutations in components of the florigen system that allowed us to fine-tune florigen:antiflorigen ratios in a manner that nature has not provided. This modified balance of flowering signals resulted in an altered meristem maturation schedule leading to a novel determinate architecture with the highest yields.

Currently, we are expanding this toolkit by integrating new alleles of uncharacterized components of the tomato florigen pathway. One example is the florigen homolog SP5G, which we identified as a promising candidate by exploiting natural variation in flowering time and shoot architecture between the domesticated tomato and its distant wild relative Solanum pennellii. The S. pennellii allele of SP5G causes delayed flowering when introgressed into domesticated tomato. We used CRISPR–Cas9 to engineer mutations in SP5G (CR-sp5g), and the resulting plants flowered faster than normal, leading to earlier fruit set and ripening. In our first field trials, we observed that CR-sp5g mutants are not compromised in important fruit quality traits such as size and sugar content. We are currently performing genetic and molecular analyses to understand the mechanisms underlying the function of SP5G, with the ultimate goal of adding this mutation, and others in related genes, to our growing florigen toolkit.
(S. peruvianum). These results suggest that subtle heterochronic shifts in meristem maturation rates are critical for determining quantitative differences in inflorescence complexity. More broadly, our results suggest divergence in the activity of specific genes and pathways that modulate meristem maturation was a common theme in the evolution of extensive inflorescence architecture diversity in the Solanaceae and beyond.

Exploring the Role of Meristem Size in the Diversity of Pepper Plant Form
Z. Lemmon

The genes and pathways that determine plant form were a recurring target of selection during the domestication of multiple crops and remain a major target for crop improvement efforts today. Although the factors contributing to plant architecture are well studied in major crops such as maize, rice, and tomato, less research has been conducted in secondary crops. One important but poorly studied secondary crop is the highly diverse hot pepper clade (Capsicum ssp.), which consists of five domesticated and more than 20 wild species. We have begun investigating shoot and inflorescence architecture variation in pepper to broaden our perspective and knowledge on agriculturally important traits in this important Solanaceous crop.

We are integrating quantitative genetic, genomic, and reverse genetics tools to investigate the molecular networks directing Capsicum meristem development and resulting shoot and flower production. Primary traits under study are shoot and flower production, which are the foundation for pepper yield. Genetic diversity within and among pepper species is being assayed with high-throughput genotyping by sequencing technology on more than 400 accessions. This extensive sequence information will allow association and population genetic studies, with the ultimate goal of identifying functional polymorphisms that will lay the foundation for future mechanistic work. Finally, in collaboration with J. van Eck (Boyce Thompson Institute), an efficient transformation protocol for pepper is being established, and CRISPR will be used to mutate known and candidate shoot architecture genes identified through quantitative genetic and transcriptomic work.

PUBLICATIONS
Plants and fission yeast provide excellent models for epigenetic mechanisms of transposon regulation, heterochromatic silencing, and gene imprinting, important for both plant breeding and human health. We are investigating the role of RNA interference (RNAi) in heterochromatic silencing in the fission yeast *Schizosaccharomyces pombe* and the model plant *Arabidopsis thaliana*, as well as continuing our work on *Zea mays*. In fission yeast, we have found that RNAi promotes DNA replication and repair, as well as histone modification, in part by releasing RNA polymerase II (Pol II) in mitotic cells. In quiescence, RNAi is required for release of RNA polymerase I and for viability in the presence of heterochromatin. In plants, we have found that the genome undergoes reprogramming of DNA methylation in pollen, guided by DNA demethylases and small RNA. Histone variants and microRNA (miRNA) contribute to reprogramming by targeting transposons, suggesting a model for imprinting, transposon control, and the origin of epialleles. Epigenetic changes in *Karma*, a transposon found in oil palm, predict fruit form and oil yield in cloned palms and help improve sustainability in environmentally sensitive tropical plantations. We continue to develop duckweeds for biofuel and by sequencing the genome and developing an efficient transformation system in the clonally propagated aquatic macrophyte *Lemna minor*. This year we said goodbye to An-Yun Chang, Kate Creasey, Yannick Jacob, Chantal LeBlanc, Almudena Molla-Morales, and Mark Donoghue, who left for positions in Boston (A.-Y.C.), Stony Brook (K.C.), Yale University (Y.J., C.L.), Spain (A.M.-M.), and Memorial Sloan Kettering (M.D.). We welcomed sabbatical visitor Dr. Daniel Grimanelli from Institut de recherche pour le développement (IRD)-Montpellier in France.

### Dicer in Action at Replication-Transcription Collisions to Protect Genome Integrity

J. Ren, S. Castel, S. Bhattacharjee, H.-S. Kim, A.-Y. Chang, R. Martienssen

Maintaining genome stability and faithfully inheriting epigenetic information are major challenges for cells. Nuclear RNAi is an important regulator of transcription and epigenetic modification; however, its function in response to these challenges and the underlying mechanisms remains elusive. Previously, we have shown that inheritance of heterochromatin through mitosis requires cotranscriptional RNAi during the DNA replication phase of the cell cycle. Yet, the spatial and temporal overlap results in competition between transcription and replication. RNAi resolves the conflict by releasing RNA Pol II and couples the spreading of heterochromatin with replication fork progression. Using a genome-wide approach, we have found that Dcr1, but not other canonical RNAi components, promotes the release of Pol II from sites of replication stress, including the 3′ end of highly transcribed genes, and, surprisingly, from antisense transcription of ribosomal RNA (rRNA) and transfer RNA (tRNA) genes. A striking example is at the subtelomeric ribosomal DNA (rDNA) repeats, in which Dcr1 is required to release Pol II to facilitate DNA replication and prevent homologous recombination, which would otherwise lead to loss of rDNA repeats, especially during meiosis. Our results reveal a novel
role for Dcr1-mediated transcription termination in genome maintenance.

**RNAi Is Essential for Cellular Quiescence**
B. Roche, R. Martienssen [in collaboration with B. Arcangioli, Institut Pasteur, Paris]

Quiescent cells play a predominant role in most organisms. We have identified RNAi as a major requirement for quiescence (the G₀ phase of the cell cycle) in *S. pombe*. RNAi mutants lose viability at G₀ entry and are unable to maintain long-term quiescence. We obtained *dcr1Δ G₀* suppressors, which mapped to genes involved in chromosome segregation, RNA polymerase-associated factors, and heterochromatin formation. We propose a model in which RNAi promotes RNA polymerase release in cycling and quiescent cells: (i) RNA Pol II release mediates heterochromatin formation at centromeres, allowing proper chromosome segregation during mitotic growth and G₀ entry, and (ii) RNA Pol I release prevents heterochromatin formation at rDNA during quiescence maintenance. Our model may account for the codependency of RNAi and histone H3K9 methylation throughout eukaryotic evolution.

**Natural Variation in the Epigenetic Regulation of Arabidopsis Retrotransposons by miRNAs**
F. de Sousa Borges, M. Donoghue, J.-S. Parent, R. Herridge, R. Martienssen

In *Arabidopsis*, miRNAs trigger 21-nt secondary small-interfering RNA (siRNA) from target transposon transcripts as a first line of defense against reactivated transposable elements (TEs). Notably, the pollen-specific miRNAs miR845a (21 nt) and miR845b (22 nt) are predicted to target the primer binding site (PBS) of retrotransposons, especially Gypsy-class retrotransposons that all use tRNA Met as a primer. Using a green fluorescent protein (GFP)-reporter construct with a single target site, we were able to show that miR845b is specifically active in Col-0 pollen, but strikingly, the same transgene was not silenced in Ler-0. We found that MIR845a is deleted from Ler and many other *Arabidopsis* natural variants, whereas MIR845b has a conserved single base pair polymorphism in the passenger sequence—changing the predicted stability of the miR845b/miR845b* duplex. Consequently, many targeted TEs are differentially regulated in Ler pollen. Our results suggest that natural variation in miRNA biogenesis might contribute to trans-regulation of retrotransposon activity in plants. We are exploring the possibility that the balance of 21-nt epigenetically activated siRNA (easiRNA) and 24-nt siRNA following fertilization might impact natural variation in reproductive hybrid barriers.

**21-nt Epigenetically Activated easiRNAs Control Retrotransposition in Arabidopsis**
S.-C. Lee, J.-S. Parent, K. Creasey, E. Ernst, R. Martienssen

In *Arabidopsis*, 21-nt easiRNAs from retrotransposon transcripts trigger posttranscriptional gene silencing. In the chromatin remodeling mutant *ddm1*, transcription of most retrotransposons is dramatically increased, but very few actually transpose. We obtained total and microsome-polyosomal RNA-sequencing (RNA-seq) data from *ddm1* and *ddm1drd6*, and found that many retrotransposon transcripts were associated with polysomes, indicating active translation. Translated proteins subsequently form virus-like particles (VLPs) in which reverse transcription takes place. We purified VLP-DNA from *ddm1* and *ddm1drd6* and identified long terminal repeat (LTR) retrotransposons using next-generation sequencing. VLP-DNA of *EVADE*, a known LTR retrotransposon, was the most abundant in both *ddm1* and *ddm1drd6*. To test whether 21-nt easiRNAs regulate movement, heterozygous *ddm1* with a low copy number of *EVADE* was reciprocally crossed to * rdr6* homozygous mutants. Wild-type, *ddm1*, and *ddm1drd6* homozygous plants were isolated in the F2 and F3 generations. When paternally inherited through *ddm1* pollen, *EVADE* copy numbers of extrachromosomal and integrated elements were significantly increased in *ddm1drd6* compared with *ddm1*. The copy number increases coincided with increased levels of *EVADE* transcripts. In contrast, maternally inherited *EVADE* elements in *ddm1drd6* showed lower levels of *EVADE* transcripts and DNA copy number. *EVADE* DNA methylation levels remained low in the reciprocally derived *ddm1drd6* mutants containing paternally or maternally inherited *EVADE* copies. Thus, paternal but not maternal silencing of *EVADE* transposition may involve easiRNA.
DNA Demethylation in *Arabidopsis* Pollen Guides Transgenerational Epigenetic Inheritance

Genome reprogramming of DNA methylation is important for transposon silencing and epigenetic inheritance, and may lead to natural epigenetic variation when it goes awry. We found dynamic regulation of DNA methylation in *Arabidopsis* pollen, where two sperm cells (SCs) and a vegetative cell differentiate from a precursor microspore via postmeiotic cell divisions. The twin SCs fertilize two female gametes, giving rise to the embryo and endosperm, whereas the vegetative cell nucleus (VN) does not transmit genetic material to the next generation. We found that targeted DNA demethylation previously observed in wild-type VN requires combined activity of the DNA glycosylases DME and ROS1, resulting in global hypermethylation observed in double mutant pollen. However, a subset of differentially methylated regions (DMRs) shows hypomethylation in mutant SCs, and overlaps with variable epialleles and DME targets in the VN, supporting the idea that targeted demethylation in the VN could regulate epigenetic silencing in the gametes or in the next generation. We are currently investigating the role of targeted DNA demethylation in pollen in guiding epigenetic reprogramming of the germline.

The *A. thaliana* Mobilome and Its Impact at the Species Level
C. LeBlanc, R.A. Martienssen [in collaboration with V. Colot, Ecole Normale Superieure, Paris]

TEs are powerful motors of genome evolution, yet a comprehensive assessment of recent transposition activity at the species level is lacking for most organisms. Using genome sequencing data for 211 *A. thaliana* accessions taken from across the globe, and by hybrid capture sequencing of TE insertions, we have identified thousands of recent transposition events involving half of the 326 TE families annotated in this plant species. We have further shown that the composition and activity of the “mobilome” vary extensively between accessions in relation to climate and genetic factors. Moreover, TEs insert equally throughout the genome and are rapidly purged by natural selection from gene-rich regions because they frequently affect genes. Remarkably, loci-controlling adaptive responses to the environment are the most frequent transposition targets observed. These findings show the pervasive, species-wide impact that a rich mobilome can have and the importance of transposition as a recurrent generator of large-effect alleles.

Whole-Genome Sequencing of *L. minor* 8627
E. Ernst, A. Molla-Morales, R. Martienssen

*L. minor* clone 8627 was among the first duckweeds for which a stable transformation protocol was shown, nearly 15 years ago. In subsequent studies, it was found to possess a high growth rate on concentrated wastewater along with a favorable protein production rate. We have assembled an initial draft of the genome sequence from >75 Gbp of Illumina polymerase chain reaction (PCR)-free, mate pair, and PacBio data, spanning ~800 Mbp—the largest duckweed genome sequenced to date. The availability of this genome sequence, together with the development of new transformation protocols and artificial miRNA constructs in *L. minor* enables rapid and precise genetic engineering of this duckweed clone.

Loss of Karma Transposon Methylation Underlies the Mantled Somaclonal Variant of Oil Palm
R. Martienssen [in collaboration with J. Ordway, S. Smith, and N. Lakey, Orion Genomics LLC, St. Louis, MO; M. Ong-Abdullah, R. Nookiah, R. Singh, E.L. Low, and R. Sambanthamurthi, Malaysian Palm Oil Board, Selangor, Kuala Lumpur, Malaysia]

Somaclonal variation arises in plants and animals when differentiated somatic cells are induced into a pluripotent state, but the resulting clones differ from each other and their parents. In agriculture, somaclonal variation has hindered the micropropagation of elite hybrids and genetically modified crops, but the mechanism responsible remains unknown. The oil palm fruit “mantled” abnormality is a somaclonal variant arising from tissue culture that drastically reduces yield, and has largely halted efforts to clone elite hybrids for oil production (Fig. 1A–C). Widely regarded as an epigenetic phenomenon, “mantling” has defied explanation, but we have identified the MANTLED locus using epigenome-wide association
studies of the African oil palm *Elaeis guineensis* (Fig. 1D). DNA hypomethylation of a LINE retrotransposon related to rice *Karma*, in the intron of the homeotic gene *DEFICIENS*, is common to all mantled clones and associated with alternative splicing and premature termination. Dense methylation near the Karma splice site (termed the Good Karma epiallele) predicts normal fruit set, whereas hypomethylation (the Bad Karma epiallele) predicts homeotic transformation, parthenocarpy, and marked loss of yield. Loss of Karma methylation and small RNA in tissue culture contributes to the origin of mantled, whereas restoration in spontaneous revertants accounts for non-Mendelian inheritance. The ability to predict and cull mantling at the plantlet stage will facilitate the introduction of higher-performing clones and optimize environmentally sensitive land resources.

**PUBLICATIONS**


Ong-Abdullah M, Ordway JM, Jiang N, Ooi SE, Kok SY, Sarpen N, Azimi N, Hashim AT, Ishak Z, Rosli SK, et al. 2015. Loss of

In Press
PLANT DEVELOPMENTAL GENETICS

M. Timmermans A. Benkovics K. Petsch A. Husbands D. Skopelitis S. Knauer

Specification of adaxial–abaxial (upper/lower) polarity in the developing leaf drives the flattened outgrowth of the leaf blade and directs the differentiation of distinct cell types within the leaf’s adaxial/top and abaxial/bottom domains. Both are important innovations in the evolution of land plants that maximize photosynthesis while minimizing water loss to the environment. In addition to being a key developmental process, adaxial–abaxial patterning is of particular interest from a mechanistic point of view and has proven to be an excellent model to study small RNA-mediated gene regulation. We previously showed that patterning of this developmental axis involves a cascade of opposing small RNAs, in which microRNA miR390 triggers the biogenesis of the TAS3-derived 21 nucleotide tasiR-ARF (auxin response factor) 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 ta-siRNA (small interfering RNA) pathway in Arabidopsis showed that tasiR-ARF functions as a mobile positional signal in adaxial–abaxial patterning. Movement of this small RNA from its defined source of biogenesis in the two uppermost cell layers of leaves creates a gradient of accumulation that dissipates abaxially. The tasiR-ARF gradient generates a sharply defined expression domain of the abaxial determinant ARF3 on the bottom side of leaf primordia, suggesting that small RNAs can establish pattern through a morphogen-like activity. This work provided the first direct evidence that small RNAs are mobile and can function as instructive signals in development, thereby revealing a novel patterning activity of small RNAs. 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

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 microRNA (miRNA) targeting ARF3 (miRARF) from different leaf-specific promoters to modulate the position, direction, and steepness of this small RNA gradient. Target gene expression is being monitored by GUS histochemistry and miR166 expression patterns will be determined by in situ hybridizations. Our results strongly support the idea that small RNA gradients have the inherent ability to generate sharply defined domains of target gene expression (Fig. 1).

Patterning via small RNA gradients is also being addressed using a reporter system that monitors the readout of a miR166 gradient. A miR166-insensitive HD-ZIPIII reporter (PHB*-YFP), which 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. Our 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. Visualization of PHB*-YFP expression patterns by confocal microscopy indicates that similar to results obtained via our miR-ARF3–ARF3-GUS system, miR166* gradients
generated in different directions in the leaf create a sharp on–off expression pattern of target expression, suggesting that this is a general patterning property of small RNA gradients.

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. We are comparing variability in leaf parameters under normal and mild stress conditions between wild-type plants and plants in which either small RNA gradient is perturbed. We are also analyzing the expression patterns of adaxial and abaxial reporters in these lines to monitor the effect of perturbing small RNA gradients on the position and uniformity of the adaxial–abaxial cell fate boundary under normal and stress conditions. The outcomes 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

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 among 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 suggest 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 performing forward genetic screens to identify factors influencing this process.

The START Domain Regulates HD-ZIPIII Activity and Organ Polarity

A. Husbands

Based on 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 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. To assess the latter, we established a single-molecule pull-down (SiMPull) technique in plants that uses a single-molecule fluorescence detection method to quantitatively interrogate protein–protein interactions. SiMPull has a number of advantages over traditional methods used to assess interactions between proteins. Most importantly, SiMPull provides a quantitative readout, can be used to determine complex heterogeneity and stoichiometry, and additionally is able to interrogate interactions between proteins and other compounds, such as nucleic acids, small molecule ligands, and lipids. Using this approach, we showed that PHB forms a heterotetrameric complex with LITTLE ZIPPER proteins that modulate their activity.

As a further component of this project, we have generated plants expressing a biotin-tagged form of the START domain that is being used to identify the endogenous ligand bound by HD-ZIPIII proteins via immunoprecipitations and mass spectrometry. These latter experiments are under way in collaboration with the CSHL proteomics facility.

Novel DICER-LIKE1 siRNAs Bypass the Requirement for DICER-LIKE4 in Development

K. Petsch [in collaboration with O. Tam, M. Hammell, CSHL; R. Meeley, DuPont-Pioneer; P. Manzotti, G. Consonni, University of Milan, Italy]

Dicer enzymes function at the core of RNA silencing to defend against exogenous RNA or as an endogenous mechanism of gene regulation. Plant DICER-LIKE4 (DCL4) performs dual functions, acting in antiviral defense, as well as in development via the biogenesis of tasiR-ARFs. These small RNAs play an essential role in the grasses and act to spatially define the expression domain of ARF3 transcription factors. However, contrary to tasiR-ARFs’ essential function in development, DCL4 proteins show strong evidence of recurrent adaptation typical of host factors involved in antiviral immunity. Here, we address how DCL4 balances its role in development with pressures to diversify in response to viral attack. We show that, in contrast to other tasiR-ARF biogenesis mutants, dcl4 null alleles condition an uncharacteristically mild phenotype, correlated with normal expression of select arf3 targets. Loss of DCL4 activity yields a class of 22-nt tasiR-ARF variants associated with the processing of arf3 transcripts into 22-nt secondary siRNAs by DCL1. Our findings uncover the presence of a novel DCL1-dependent siRNA pathway that bypasses the otherwise adverse developmental effects of DCL4 mutations (Fig. 1). This novel pathway is predicted to have important implications for DCL4’s role in antiviral defense by reducing the selective
constraints on DCL4 and allowing it to diversify in response to viral suppressors.

A High-Resolution Gene Expression Atlas for the Maize Shoot Apex

S. Knauer [in collaboration with M. Javelle, M. Scanlon, Cornell University; G. Muehlbauer, University of Minnesota; J. Yu, Kansas State University; P. Schnable, Iowa State University]

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. Using this extensive data set, we were able to identify genes that mark specific cell types or are differentially expressed between distinct tissue types. Using CAST clustering, we further identified genes that show dynamic expression profiles during the transition from a stem cell to an organ primordium. For instance, we identified numerous genes that specifically mark the stem cell domain of the meristem, and several genes that mark the presumptive organizing center. Most notably, our analysis identified unique transcription factor signatures that, next to the interplay of hormones, distinguish stem cells from their differentiating descendants within the SAM. 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 mutants affecting meristem maintenance and leaf morphology. Overall, our findings present a novel approach to modulating plant architecture by manipulating master regulators of cell identity.

PUBLICATIONS


In Press


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.

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 on 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.

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 10 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 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 first plant genome sequenced) and the fission yeast Schizosaccharomyces pombe, as well as the human genome and other important genomes. McCombie’s group is currently involved in several important projects to re-sequence 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. They are also looking for genes that contribute to cancer progression using whole-genome sequencing or a method called exome sequencing, which they developed with Greg Hannon to look at mutations in the regions of the genome that code for proteins.

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 the 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—a single, integrated cyber-“knowledgebase” for plants and microbial life.
GENE NETWORK META-ANALYSIS

J. Gillis  S. Ballouz  M. Crow

Research in the Gillis lab centers on using computational methods to understand gene function. As data sets characterizing genes grow in size and complexity, novel means of interpreting that data become essential. Because much of that preexisting data is readily available, one way to learn more from it is to have methods that can integrate results across hundreds or even thousands of independent prior experiments. This type of data integration, called meta-analysis, allows us to probe functional properties of genes at a scale that is otherwise impossible, with a corresponding increase in precision. A popular means of integrating disparate data is to treat each data type as a means of linking genes within a common network. The expression profiles of genes across thousands of samples are a particularly fruitful means of finding similarities among genes, or coexpression links, which are critical to their biological properties. This approach, often called “guilt by association,” is embedded in everything from uncovering novel molecular phenotypes or mechanisms of disease to learning features of cell identity. In the Gillis lab, we are developing coexpression meta-analytic methods and software that improve both the sophistication and breadth of data available for determining functional convergence among disease-causal genes. Broadly, our research can be divided into methods development and our own research applications, often performed in collaboration with other labs to test computational predictions experimentally.

Effect Size Meta-Analysis

Disagreements over genetic signatures associated with disease have been particularly prominent in the field of psychiatric genetics, creating a sharp divide among disease burdens attributed to common and to rare variation, with study designs independently targeting each. Meta-analysis, including using underlying data, within each of these study designs is routine, but no method for combining the results across study designs exists. Traditional meta-analytic approaches are not calibrated to detect functional convergence within these data because significant disease gene associations from the different study designs arise in quite different ways, with quite different null hypotheses. In this work, we develop a general solution that integrates the disparate genetic contributions constrained by their observed effect sizes to determine functional convergence in the underlying architecture of complex diseases, which we illustrate using autism spectrum disorder (ASD) data.

Our approach looks not only for similarities in the functional conclusions drawn from each study type individually but also those that are consistent with the known effect sizes across these studies. We name this the “functional effect size trend,” and it can be understood as a generalization of a classic meta-analytic method, the funnel plot test. Because this allows us to combine many different studies, and to do so in a structured way, it has the potential to find overlapping functional signals that would otherwise be impossible to see. We took candidate disease gene data from multiple ASD studies across thousands of individuals (~7000) and study designs, including whole-exome sequencing and genome-wide association studies. We split the candidate genes by variant class (common and rare) and effect size (low to high) into 14 gene sets, in the range of 20 to 100 associated genes per set, and performed functional analyses, controlling for set size. We detected remarkably significant trends in aggregate with 20 individually significant properties (FDR [false discovery rate] <0.01), many in areas researchers have targeted based on different reasoning, such as FMRP (fragile X mental retardation protein) interactor enrichment (FDR ~0.006). We are also able to detect novel technical effects and we see that network enrichment from protein–protein interaction data is heavily confounded with study design, arising readily in control data. We perform a number of downstream analyses, including tests for robustness and genome-wide rankings of disease association. Our focus is on ASD and gene-level associations, but the method can
be extended to other diseases and other types of tests as data permits (e.g., phenotype screening, grouping of disorders, variants within studies).

**Single-Cell Coexpression Networks**

In a close collaboration with the Huang lab at CSHL, we have been exploring the basis of cellular identity and pathways underlying it using single-cell expression data. Single-cell RNA sequencing has emerged as a powerful technique to explore heterogeneity among cells, and it has now been applied to many human and mouse tissue types at multiple stages of development, including the lung, spleen, brain, retina, and lumbar dorsal root ganglia, among others. Because these studies are seeking to discover novel cell identities, they have tended to use unsupervised clustering of cells as defined by their expression profiles. Once novel groupings are determined, the researchers can assess them in more traditional ways (i.e., with differential expression), often with the intent of finding novel biomarkers for the subtypes identified. Whereas it is straightforward to test enrichment of existing pathways based on these gene sets, it is far less common to search for novel pathways in these newly defined cells. Coexpression analysis is particularly well-suited for this task and, in recent work, we have developed a host of meta-analytic methods specifically targeted toward single-cell expression data. Single-cell RNA sequencing is known to have specific and distinctive technical artifacts and, so, uncovering novel biology within it requires approaches tailored both to avoid those confounds and to exploit its greater transcriptional specificity. Broadly, our methods are distinguished from others both by focusing on coexpression signatures, rather than individual genes, and also by exploiting preexisting knowledge as to the function of genes—a form of machine learning.

**Machine Learning Functional Properties of Genes**

Increasingly, biologists have turned to computational methods to sift through the vast array of preexisting genomics data for validation that a candidate variant has a molecular role in the phenotype of interest or to prioritize a candidate as disease causal. These computational methods usually fit under the rubric of “machine learning,” using network data that represent the interaction of genes or their products.

Using 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. This is a critically important problem in evaluating disease candidate variants, many of which involve otherwise unstudied genes. 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 developed a computational infrastructure that allows us to implement representative samples of cutting-edge machine learning algorithms. These algorithms can then be customized to specific biological problems and when benchmarked against prior data, deliver very high levels of performance. Moreover, we learn more by combining them through meta-analysis than we do from their individual results, as is often the case in machine learning. Because we now have in-house versions of multiple algorithms, we can explore variance and data dependencies in a way not typically possible, leading to several useful heuristics for meta-analysis, which we have recently published.

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.

**Ligand-Binding Networks**

With the development of large-scale target-ligand databases and new chemoinformatics metrics, it is now feasible to directly correlate ligand similarities among proteins to their bioinformatic or proteomic similarities. We hoped that by investigating this alternate source of network data, new bridging ligands may be found for targets whose activity is perturbed in disease. In collaboration with the Shoichet lab at University of California San Francisco, we calculated similarities for 1131 proteins, each of which had known ligands, and compared ligand-based similarities to those derived from sequence, protein–protein interaction networks, and coexpression networks. Surprisingly, the proteomic associations were often orthogonal to the ligand-based associations; two targets that were intensely related by protein–protein interaction or by coexpression only rarely recognized similar ligands; and most pairs of targets that bound similar ligands were unrelated proteomically. Even sequence similarity was an unreliable predictor of shared ligand recognition among targets; most related sequences had only a 55% chance of sharing similar ligands, and 29% of the targets that recognized similar ligands had no significant sequence similarity. Still, there were regions of high proteomic similarity that overlapped with high ligand similarity and that were coannotated for diseases. We sought single ligands that could bridge disease-linked targets, where no such bridging ligand was known. On experimental testing, we found such bridging ligands for seven pairs of targets, previously unknown to share ligands, but linked proteomically in neuropsychiatric diseases.

**Improved Quality Control for RNA-seq**

[In collaboration with A. Dobin, CSHL]

Recent analyses of RNA-seq (RNA sequencing) have emphasized the value added by experimental designs with more samples, permitting better estimation of biological and technical variability. Unfortunately, this does not automatically translate into an experimental design decision for more replicates (biological or technical), largely because of the relatively high cost of RNA-seq. Study design is further complicated by complex dependencies on the platform chosen, library preparation, and normalization methods; all these factors affect the concordance of downstream differential expression analysis. Although researchers have to be aware of the pitfalls that can arise when designing their experiment, they receive only modest feedback in the experimental data itself as to whether their choices were successful. Although many optimized protocols or best practices exist, there are few means for assessing the quality of a given transcriptomic study, particularly outside of purely technical concerns and when the focus is on novel biology.

In our recent work, we have shown a straightforward but surprisingly powerful method to measure replicability by modeling the effects of noise within observed coexpression. Because of the breadth and extent of coexpression, it provides a sensitive, yet general, means of performing quality control. We begin our analysis by performing some sample and replicate-based analyses of RNA-seq quality to establish general properties, then we show how common heuristics for quality control appear in a coexpression-based framework and, finally, we provide a means of directly quantifying this by disruption of known coexpression pairs. By providing a direct quality control measure, we hope to make it straightforward to customize experiments to do better than a general heuristic—such as discarding all low-expressing genes—would normally permit.

We have been working on using these quality control statistics to refine RNA-seq alignment and technical efficacy.

**Heuristics for the Interpretation of Gene Sets**

One branch of our research tackles an almost philosophical problem of broad impact in bioinformatics: How can we evaluate methods in which no gold standard exists? This is a particular problem when the bioinformatics methods are targeted to functional interpretation, in which not only is a gold standard challenging, but knowing whether method performance will generalize is a major impediment.
The most striking example of this problem occurs in function enrichment methods, which are ubiquitous, diverse, and very hard to assess.

We propose a solution to this problem by looking to assess not whether enrichment results are correct, but whether they meet basic criteria for coherence. In particular, we show that assessing algorithm outputs for uniqueness and robustness is highly useful across a variety of problems. A substantial part of the work involves formalizing a test for uniqueness or robustness that can be applied to any enrichment method, but ultimately the principles map to surprisingly intuitive biological properties in the form of an assessment of the role of multifunctional genes within study results.

Gene set analysis to translate gene lists into enriched functions is among the most common bioinformatic methods, yet few would advocate taking observed \( p \)-values at face value. Not only is there no agreement on the algorithms themselves, there is no agreement on how to benchmark them. We evaluated the robustness and uniqueness of enrichment results. We showed that both properties turn out to be closely linked to multifunctionality, defined as the number of annotated functions a gene has. We showed that multifunctional genes are more likely to appear in genomics study results and drive the generation of biologically nonspecific enrichment results because of their overlaps in annotations. Additionally, individual multifunctional genes easily generate apparent, yet highly fragile, significance. By providing a means of benchmarking when enrichment analyses report nonspecific and nonrobust findings, we are able to assess where we can be confident in their use. We found that gene set enrichment methods that correct for biases are far more likely to return robust and unique results. We provided a software implementation for multifunctionality assessment in ErmineJ, but our general approach can be adapted to any enrichment method.

**PUBLICATIONS**


*In Press*

Human ENCODE Project


ENCODE project (Phase III) has completed its third year. The production effort this third year yielded 136, 122, and 136 RNA-depleted long (>200 nt) and short (<200 nt) RNA and RAMPAGE (RNA Annotation and Mapping of Promoters for the Analysis of Gene Expression) data sets, respectively. These efforts include the profiling of primary and immortalized cell lines, embryonic cell lines differentiated into smooth muscle, hepatocytes, neural progenitor cells, induced pluripotent stem cells (iPSCs) differentiated into bipolar spindle neurons, and 20 to 30 postmortem organ tissues obtained from two male and female donors. These and all data generated for the transcriptome portion of the ENCODE project are available at https://www.encodeproject.org/search/?type=experiment&lab.title=Thomas%20Gingeras,%20CSHL. Comparative analyses of these data sets have yielded insights into several biological and computational areas, including the following.

- Comparison of RNA-sequencing (RNA-seq) data derived from human organ/tissue versus constituent primary cells. We analyzed the transcriptome of 53 human primary cell lines and the gross sectioning of the organs from which they were derived. Gene expression and splicing patterns of transcripts detected both in the primary and organs were studied. It was observed that similar cell types share very similar gene expression and splicing profiles independent of the tissue/organ of origin. The strong similarity exhibited by expression and splicing profiles dominates the influence of the tissue environment from which the cells were derived. The major cell types represented in the experiments were endothelial cells, epithelial cells, melanocytes, and more heterogeneous groups, including smooth muscle cells (SMCs), mesenchymal stem cells (MSCs), and fibroblasts. We identified those genes whose expression constitutes a signature specific to each cell type group. Although the majority of these genes were composed mostly of protein-coding genes, expression of long noncoding RNAs (lncRNAs) was observed; these are conserved between human and mouse, and contributed to the cell type clustering. These are now the subject of functional studies. A total of about 100 novel transcripts that seem to be full length at the 5′ end (aligning to CAGE data), and whose positions, with respect to annotated transcripts, reveal three main classes: 5′ extensions, antisense transcripts, and novel intergenic transcripts.

- Finding the dominant contribution of organs versus species as a dominant influence affecting the variation of gene expression. There has been an ongoing discussion about whether RNA-seq samples from different organs and species should cluster by organ or by species. A linear model for the gene expression was used to assess the relative contribution to the clustering of expressed genes produced by organs versus species. Applying this model to six organs from seven different species (data from Barbosa-Morais et al., Science 338: 1587 [2012]), the results identified two groups of genes in which one was highly variable across organs and minimally variable across species, and one that was highly variable across species and minimally variable across organs. We then independently used these two sets of genes to analyze our own RNA-seq data sets. Again, two different clusterings were observed: one by organ and one by species, with the species gene set being the strongest. These results were published in Lin et al. (Proc Natl Acad Sci 111: 17224 [2014]).

- Principled definition of “housekeeping” genes: identification of genes whose range of variation in levels of expression is highly constrained across mouse,
human, and other species. Mice have been a long-standing model for human biology and disease. As reported in Pervouchine et al. (2015), using our RNA-seq data sets, we reported transcriptional profiles of a large and heterogeneous collection of mouse tissues, augmenting the mouse transcriptome with thousands of novel transcript candidates. Comparison with transcriptome profiles in human cell lines reveals substantial conservation of transcriptional programs and uncovers a distinct and large (in mass) class of genes whose levels of expression have a constrained range of variation. This property of constraint in the range of variation appears to have evolved early in vertebrate evolution. This core set of genes captures a substantial fraction of the transcriptional output of mammalian cells and participates in basic functional and structural housekeeping processes common to all cell types. Perturbation of these constrained genes is associated with significant embryonic lethality and cancer. Evolutionary constraint in the range of gene expression levels is not reflected in the conservation of the genomic sequences but is associated with conserved epigenetic marking, as well as with the characteristic posttranscriptional regulatory program, in which subcellular localization and alternative splicing play comparatively large roles. Identification of this property for this group of thousands of genes provides a principled method that can be applied to identifying and defining the membership of the housekeeping gene set.

**Database and Software Development**

C. Davis, A. Dobin, C. Zaleski

Software and database development has been an integral part of the Functional Genomic program of our laboratory. The computational output of this effort has been motivated not only by the experimental requirements that have arisen as part of the ENCODE project, but also by the need for tools to be developed and freely available to the general scientific community, and can be used for any genomics projects seeking similar genomic mapping and characterization goals. This year, three major software projects were completed and released.

- Pipeline for splicing analyses. In collaboration with Rogeric Guigo’s laboratory at the CRG in Barcelona, an integrated pipeline for splicing analysis (IPSA) was developed to provide a uniform and standardized processing protocol for all types of RNA-seq data, including stranded and unstranded sequencing data and data with or without bioreplicates. IPSA counts split reads corresponding to annotated and novel splice junctions. Shannon entropy is used to control for the support level by distinct staggered positions and excludes artefactual large counts. Splice junctions receive a number of descriptors reflecting the annotation status, splice site nucleotides, and read counts in support of splicing, as well as local intron retention at each splice site. These descriptors are used to compute the PSI (percent-spliced-in) metric for exons and introns and coSI (completeness of splicing index). The pipeline was recently extended to estimate the abundance of mini-exons (exons shorter than the read length that is supported by splice junctions at each end) in GTEx (Genotype-Tissue Expression) transcriptomics data. These results were published in Méle et al. (*Science* 348: 660 [2015]).

- Pipeline for detecting chimeric transcripts: ChimPipe is a flexible pipeline for the detection of chimeric transcripts from short RNA-seq reads created as a collaboration between our laboratory and the Guigo laboratory (CRG, Barcelona). Starting from either raw or mapped reads, this pipeline identifies exonic junctions connecting two different genes in the genome based on both split-mapping and paired end reads. Different filtering steps (excluding junctions among genes with high similarity, requiring a certain number of staggered reads supporting the junctions) ensure a rather low false-positive rate while maintaining a reasonably high sensitivity. The method is currently being compared with other tools. The tool is already available to the community through github (https://github.com/Chimera-tools/ChimPipe), together with an extensive documentation (http://chimpipe.readthedocs.org/en/latest/).

- RNA-seq universal pipeline development and implementation at the ENCODE Data Coordination Center (DCC). Our laboratory, which is part of the ENCODE RNA Working Group, has continued to be active in working toward getting universal pipelines through which RNA-seq data could be processed by the ENCODE consortium and the general scientific community. This has been a collaborative effort between experimental ENCODE RNA-seq data producers and computational folks responsible for development of the programs and
pipelines. In the last year, the pipelines have been finalized and delivered to the ENCODE DCC to be implemented and run by the DCC. These pipelines will take FASTQ files for long RNA-seq, short RNA-seq, and RAMPAGE and map them, generating BAMs and bigwigs. Quantifications for long RNA-seq data will also be produced. All of these files, along with the programs/pipelines through which they were generated, are publicly available and also implemented on DNANexus, where they can be run by anyone.

- Development of Precision-STAR: unbiased allele-aware mapping of RNA-seq reads to personal genomes. Being a part of the drive toward precision medicine, personal genomics holds the promise for identifying genetic predispositions for common diseases, diagnoses, and management of hereditary disorders, characterization and individual treatment of cancer, and genotype-guided drug research and administration. Precision-STAR, an extension of the RNA-seq aligner STAR, uses genotype information to produce more accurate allele-aware mapping of RNA-seq data. Unlike many other approaches, Precision-STAR incorporates the variants in the form of variant call format (VCF) files directly at the mapping step and produces the allele-specific alignment in one passage. Each short variant (single-nucleotide polymorphism [SNP] or short indel) is appended to the reference genome together with its surrounding sequence, while keeping track of its genomic locus and allele. In the seed search phase, the reads are mapped to both the reference genome sequence and the short variant sequences, which eliminates reference mapping bias. In the seed stitching phase, the seeds from the reference genome and short variant sequences are used together to construct the final highest-scoring alignment. Because of the favorable logarithmic scaling of the STAR’s mapping speed with the reference length, the addition of the short variant sequences does not significantly reduce the mapping speed.

The end product of the Precision-STAR mapping run includes haplotype information (maternal, paternal, undetermined) for each read. It also produces allele-specific quantifications at the level of individual short variants, as well as for entire genes and transcripts. Precision-STAR is also capable of haplotype-aware mapping and allele-specific expression (ASE) computation even without existing genotype information using the following algorithm. In the first pass, the reads are mapped to the reference genome, and the short variants are detected. In the second pass, the short variants are added to the reference genome as described above, and the reads are remapped to the on-the-fly generated personal genome. This approach is accurate for medium and highly expressed genes.

Using simulated and real data, we show that our approach eliminates most of the reference mapping biases and achieves high fidelity in detecting ASE. To show superior efficiency of Precision-STAR, we reprocessed the ENCODE and GEUVADIS data to produce bias-free personal genome alignments and ASE.

- Sequencing of full-length RNA transcripts on the Oxford Nanopore platform. The sequencing of transcripts longer than a few hundred nucleotides presents multiple challenges that cannot be resolved by the existing high-throughput sequencing technologies. The computational approaches that assemble transcripts from the RNA-seq data often yield inaccurate results, because of the inherent lack of long-range connectivity information in short reads. Other confounding challenges are the very large range of copy number variation for individual RNA transcripts, posttranscriptional RNA processing, and sequence similarity of alternative isoforms and their paralogs. Despite the relatively high base calling error rates and relatively low throughput/productivity, the long-range sequencing approach such as Oxford Nanopore Technology (ONT), combined with novel mapping strategies, holds the promise of providing full-length connectivity information for long transcripts.

Here, we report the application of the ONT MinION portable instrument for sequencing cDNAs from human cell lines that have been previously extensively characterized using short-length Illumina reads. Multiple aligners and mapping strategies were assessed to develop the optimum computational pipeline that uses short Illumina reads to improve mappability of the long but error-prone ONT reads and allows for detection of both annotated and novel full-length transcripts. We generated approximately 40,000 two-directional (2D) reads per MinION flow cell, of which ~60% can be mapped to human
transcriptome with ~1-kb median mapped length and ~22% median error rate. More than 10,000 annotated transcripts were detected, and the ONT reads showed good precision of the transcription start site recovery (deviation ~ 200 nt) and very high precision of the transcription termination site recovery (deviation < 50 b). In addition, we identified more than 1000 previously unannotated transcripts, of which approximately 700 were novel extensions of annotated 5′/3′ untranslated regions (UTRs), and approximately 600 were novel splicing isoforms. We also compared the ONT results with those obtained from the Pacific Biosciences sequencer for the same RNA sample, contrasting the strengths and weaknesses of the two long-read technologies. We show that both technologies can be used in conjunction with short Illumina reads to augment and/or correct the annotations of thousands of human genes.

### Extracellular Vesicles (EVs) Are Centers for Sorting and Processing of Various Types of RNAs

S. Chakrabortty, A. Prakash

A comprehensive characterization of the nature and diversity of RNAs packaged into extracellular vesicles (EVs) derived from nine different primary and transformed cell types has been performed, revealing a reproducible, nonrandom, and cell type–specific sorting of the different types of RNAs into EVs by the cell of origin. This sorting of specific RNAs into EVs is a dynamic process that varies based on the physiological conditions of the source cells. As seen previously, EV RNAs are capable of eliciting a transcriptional response by the recipient cells, as well as corresponding striking cellular phenotype (Chakrabortty et al. 2015). Recently, efforts in the laboratory have focused on transfer RNA (tRNA) fragments that are found within EVs to determine whether these RNA biotypes are also subject to selective processing by EVs. Our latest results indicate that a selective enrichment of processed fragments whose parent tRNA is transcribed from specific tRNA genes are differentially distributed among a cell of origin and its vesicles. This enrichment appears to be independent of the anticodon marked by the particular gene and instead is based on sequences present within the 5′ and 3′ halves of the molecules. Further studies of the relative distributions of 3′ halves, 5′ halves, and full-length tRNA within cells and vesicles indicate that for some tRNA transcripts, the 5′ halves of that tRNA are exclusively detected in vesicles and not observed in their parent cells. These observations suggest the possibility that these 5′ fragments were processed from full-length tRNA precursor molecules within EVs for export outside of the parent cell.

### Mechanisms of RNA Processing in EVs

G. Nechooshtan

Although it has been shown that this RNY5 RNA is present in EVs as several distinct processed species and that processed forms of RNY5 can induce apoptosis when administered to recipient primary cells, little is understood concerning the formation, sorting, and enrichment of these processed species. Recently, deciphering the molecular path that leads to sorting of RNY5 into EVs and its processing into distinct fragments has proceeded by the use of an RNA–protein UV cross-linking approach. This approach has been used to find the binding partners of the full-length RNY5. In addition to known Y RNA-binding counterparts (Ro60, La, Nucleolin), RNY5 has been observed to be bound by several other RNA-binding proteins (HNRNP H1, HNRNP F, CUGBP1, and PCBP1). In addition, chromatographic separation approaches have been used to isolate the factor(s) responsible for processing of the RNY5 transcript. In collaboration with Jon Ipsaro from the Joshua-Tor laboratory, optimized purification steps are being developed to identify the processing protein in these chromatographic fractions.

### PUBLICATIONS


Our lab focuses on analyzing human genetic variation and its role in severe neuropsychiatric disorders and rare diseases, including intellectual disability, autism, and schizophrenia. By recruiting large groups of related individuals living in the same geographic location (e.g., Utah), we 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, we look for mutations that segregate with syndromes in the various populations, and we undertake comprehensive functional studies of many of the newly identified mutations. There are approximately 12 billion nucleotides in every cell of the human body, and there are approximately 25–100 trillion cells in each human body. Given somatic mosaicism, epigenetic changes, and environmental differences, no two human beings are the same—especially as there are only about seven billion people on the planet. One of the next great challenges for studying human genetics will be to acknowledge and embrace complexity. Every human is unique, and the study of human disease phenotypes (and phenotypes in general) will be to acknowledge and embrace complexity. Every human is unique, and the study of human disease phenotypes (and phenotypes in general) will be to acknowledge and embrace complexity.

The Amino-Terminal Acetylation of Proteins
M. Doerfel, Y. Wu, J. Crain

More than 85% of human proteins are acetylated at their amino-terminal amino group; hence, amino-terminal acetylation (NAT) is one of the most abundant modifications of eukaryotic proteins. Despite its discovery more than 30 years ago, very little is known about the cellular effects/functions of this modification. In humans, six distinct NATs catalyze the transfer of an acetyl group from acetyl-CoA to the amino-terminal amino group of their specific target proteins. The major human acetyltransferase, NatA, consists of an auxiliary subunit, Naa15, and a catalytic active subunit, Naa10. We have previously described two families with a lethal X-linked disorder of infancy called Ogden syndrome. This disorder comprises a distinct combination of an aged appearance, craniofacial anomalies, hypertonia, global developmental delays, cryptorchidism, and cardiac arrhythmias. Using X chromosome exon sequencing, we identified a c.109T>C (p.Ser37Pro) variant in Naa10 as contributing to this disease. Biochemical analysis and immunoprecipitation assays in combination with liquid chromatography–mass spectrometry (LC/MS) showed a reduced catalytic capacity and revealed an impaired binding of the S37P mutant toward specific interaction partners, including Naa15 and Naa50. Analysis of the amino-terminal acetylome of patient cells revealed a decreased acetylation of a subset of NatA substrates, indicating that a reduced binding capability and an affected enzymatic activity of the Naa10 S37P mutation is a prominent feature in Ogden syndrome. Characterization of NAA10/NAA15 knockout yeast strains revealed various phenotypes, including growth defects at elevated temperatures and altered sensitivity toward cytotoxic stresses. These effects could be rescued by overexpressing human wild-type (wt) Naa15/Naa10 from plasmids; however, overexpressing mutant Naa15/Naa10 S37P only partially rescues these effects. Interestingly, introduction of both human Naa15/Naa10 wt and S37P mutant into the endogenous locus of the corresponding yeast genes failed to...
reverse the effects. We also continued our efforts with establishing induced pluripotent stem cells (iPSCs) from skin fibroblasts from one of the boys with Ogden syndrome, and we have also established knockout mice for NAA10 and knock-in mice containing the mutation of interest in NAA10. Ongoing work will focus on characterizing the cells and mice.

TAF1 Syndrome: Characterization and Analysis of an Idiopathic Intellectual Disability Syndrome
J. O’Rawe, Y. Wu [in collaboration with H. Fang, J. Jiménez-Barrón, CSHL; E. Yang, Boston; A. Rope, Oregon; J. Swensen, Arizona; R. Robison, Utah; K. Wang, California]

This past year, we expanded and published our study on a new X-linked genetic syndrome associated with mutations in TAF1 and manifesting with global developmental delay, intellectual disability (ID), characteristic facial dysmorphology, generalized hypotonia, and variable neurologic features, all in male individuals. Simultaneous studies using diverse strategies led to the identification of nine families with overlapping clinical presentations and affected by de novo or maternally inherited single-nucleotide changes. Two additional families harboring large duplications involving TAF1 were also found to share phenotypic overlap with the probands harboring single-nucleotide changes, but they also showed a severe neurodegeneration phenotype. Functional analysis with RNA-seq for one of the families suggested that the phenotype is associated with down-regulation of a set of genes notably enriched with genes regulated by E-box proteins. In addition, knockdown and mutant studies of this gene in zebrafish have shown a quantifiable, albeit small, effect on a neuronal phenotype. Our results suggest that mutations in TAF1 play a critical role in the development of this X-linked ID syndrome.

Development of Comprehensive Whole-Genome Sequencing Analysis Pipelines
J. O’Rawe, Y. Wu [in collaboration with H. Fang, L. Jiménez-Barrón, M. Schatz, G. Narzisi, CSHL; K. Wang, California; and M. He, Wisconsin]

We continued developing various bioinformatics approaches for the analysis of exome and whole-genome sequencing (WGS) data. For example, in one project, we showed that the accuracy of detection of small insertions and deletions (indels) is greater when using whole-genome sequencing versus exon capture and sequencing. We also calculated that $60 \times$ WGS depth of coverage from the Illumina HiSeq platform is needed to recover 95% of indels detected by Scalpel. Although this is higher than current sequencing practice, we proposed that the deeper coverage may save total project costs because of the greater accuracy and sensitivity. Finally, we investigated sources of INDEL errors (e.g., capture deficiency, polymerase chain reaction [PCR] amplification, homopolymers). Over the past 12 months, we reported the results of several other ongoing bioinformatics projects as well, as shown in the publications listed below. For example, we developed SeqHBase, a big data-based toolset for analyzing family-based sequencing data to detect de novo, inherited homozygous or compound heterozygous mutations that may be disease contributory. We showed SeqHBase’s high efficiency and scalability, which is necessary as WGS and whole-exome sequencing (WES) are rapidly becoming standard methods for studying the genetics of familial disorders. We also published an opinion piece regarding the current state of uncertainty about quantification in DNA sequencing applications, and we proposed methods that can be used for accounting and propagating these errors and their uncertainties through subsequent calculations.

Genome-Wide Variant Analysis of Simplex Autism Families with an Integrative Clinical-Bioinformatics Pipeline
J. O’Rawe, Y. Wu [in collaboration with L. Jiménez-Barrón, M. Yoon, H. Fang, and I. Iossifov, CSHL]

Autism spectrum disorders (ASDs) are a group of developmental disabilities that affect social interaction and communication and are characterized by repetitive behaviors. There is now a large body of evidence that suggests a complex role of genetics in ASDs, in which many different loci are involved. Although many current population-scale genomic studies have been demonstrably fruitful, these studies generally focus on analyzing a limited part of the genome or use a limited set of bioinformatics tools. These limitations preclude the analysis of genome-wide perturbations that may contribute to the development and severity of ASD-related phenotypes. To overcome
these limitations, we developed and used an integrative clinical and bioinformatics pipeline for generating a more complete and reliable set of genomic variants for downstream analyses. Our study focused on the analysis of three simplex autism families consisting of one affected child, unaffected parents, and one unaffected sibling. All members were clinically evaluated and widely phenotyped. Genotyping arrays and whole-genome sequencing were performed on each member, and the resulting sequencing data were analyzed using a variety of available bioinformatics tools. We searched for rare variants of putative functional impact that were found to be segregating according to de novo, autosomal-recessive, X-linked, mitochondrial, and compound heterozygote transmission models. The resulting candidate variants included three small heterozygous copy number variations (CNVs), a rare heterozygous de novo nonsense mutation in MYBBP1A located within exon 1, and a novel de novo missense variant in LAMB3. Our work showed how more comprehensive analyses that include rich clinical data and whole-genome sequencing data can generate reliable results for use in downstream investigations.

**KBG Syndrome Involving a Single Base Insertion in ANKRD11**


KBG syndrome is a rare autosomal-dominant genetic condition characterized by neurological involvement, macrodontia, and distinct facial, hand, and skeletal features. More than 70 cases have been reported; however, it is likely that KBG syndrome is underdiagnosed because of lack of comprehensive characterization of the heterogeneous phenotypic features. We describe the clinical manifestations in a male referred at 11 years of age, who showed symptoms including epilepsy, developmental delay, distinct facial features, and hand anomalies, without positive genetic diagnosis. Subsequent exome sequencing identified a novel de novo heterozygous single base pair insertion (c.6015dupA) in ANKRD11, which was validated by Sanger sequencing. We predict that this insertion leads to a premature stop codon and loss of function in ANKRD11, thereby implicating it as contributing to the proband’s symptoms and yielding a molecular diagnosis of KBG syndrome for the case.

**SCN8A Mutation in Child Presenting with Seizures and Developmental Delays**


The SCN8A gene encodes the Nav1.6 neuronal voltage-gated sodium channel α subunit. Mutations in this gene have been associated with early infantile epileptic encephalopathy type 13. With the use of whole-exome sequencing, a missense mutation was identified in a 4-yr-old female who initially showed symptoms at the age of 5 mo after she received routine vaccinations. Determining the molecular etiology of this proband’s epileptic encephalopathy has improved her management and treatment.

**Expanding Collection and Sequencing of Other Rare Genetic Syndromes**

J. O’Rawe, Y. Wu [in collaboration with H. Fang, R. Robison, Utah; K. Wang, California; A. Rope, Oregon]

We continue to meet and collect many families in Utah and elsewhere with very rare, idiopathic genetic syndromes. The total number of DNA samples collected to date is approaching 2000, and this includes detailed phenotyping information. Some of these samples have undergone exome or whole-genome sequencing, and we are currently analyzing 18 whole genomes and 50 exomes generated as part of this project. This includes the ongoing analysis of nine whole genomes from a pedigree with Prader–Willi syndrome (PWS), hereditary hemochromatosis, familial dysautonomia (FD), and Tourette syndrome.

**Collaborating on the Genetics of Tourette Syndrome**

This work was done in collaboration with the Tourette Syndrome Association International Consortium for Genetics.

The PI continues to collaborate on this international effort to understand the genetics of Tourette syndrome (TS). Psychiatric comorbidity is common in TS; when present, these conditions typically cause more distress and impairment than do tics. High rates of attention-deficit/hyperactivity disorder (ADHD) and obsessive–compulsive disorder (OCD) are well
documented and thought to be core components of the TS phenotype; however, few studies have fully characterized other comorbidities. We therefore continue to characterize the prevalence and impact of psychiatric comorbidity in a large sample of individuals with TS and their family members.

PUBLICATIONS


We continue our lab’s work of developing and deploying new technologies to better understand the correlation between structure and function in the genomes of plants and animals. A major effort this past year has been in improving the resolution of sequencing by developing methods to sequence genomes using extremely long reads generated by Pacific Biosciences (PacBio) and Oxford Nanopore instruments. Although still not cost-effective, these methods are providing extremely high-resolution genome sequences, which are not possible to obtain with other methods. In the previous year, we used this technology for a small plant genome (rice), and in 2015, we completed sequencing the genome of a human cancer cell line using this technology. We found details and information present in this genome using PacBio technology that was not observed using other methods. We also have used more standard sequencing methods to sequence the genomes and exomes of patients with psychiatric disorders. These were compared with genomes of control individuals, and interesting findings have been made that are being pursued further. In particular, we sequenced the genes that interact with DISC1 (Disrupted in schizophrenia 1) in a large number of patients and controls. We previously reported on the exome sequencing and primary analysis of 1135 bipolar cases and 1142 controls that we deemed the RareBLISS study. In addition to refining previous variant, gene burden, gene set, and pathway tests for this data set, in 2015 we analyzed the impact of noncoding variants that were targeted for approximately 1400 genes related to the synaptic density by our custom capture probe set. We also incorporated a polygenic load analysis using the Psychiatric Genomics Consortium (PGC) genome-wide association study (GWAS) data to address any possible interaction between the burdens of common and rare variants. Although there were some suggestive findings, none of the analyses yielded a statistically significant result after correction. We will work to finalize a manuscript for the case-control study in 2016.

The family study focused on identifying rare (<1% MAF [minor allele frequency]) segregating variants in eight families with at least four affected individuals sequenced per family. We selected rare variants that were either loss of function (nonsense, frameshift, or splice site) or predicted to be damaging by at least one of five algorithms (SIFT, PolyPhen-2 HVAR, PolyPhen-2 HDIV, LRT, and MutationTaster) from the ANNOVAR annotation. A total of 84 variants in 82 genes fitting these criteria were found. We then checked for replication in the RareBLISS case-control cohort,
using both variant level and gene burden tests. A meta-
analysis was also performed using two large ethnicity-
matched bipolar data sets from Sweden (1022 cases
and 2220 controls) and the BRIDGES study (1388
cases and 1412 controls). Although no variant or
gene was statistically significant after correction, gene
set enrichment tests showed an overrepresentation of
genes previously identified in de novo studies of autism
and schizophrenia, supporting the growing evidence of
genetic overlap across psychiatric disorders. A manu-
script detailing our findings was submitted for publica-
tion in The Journal of the American Medical Association
(JAMA) Psychiatry and will be published in 2016.

Whole-Genome Analysis of Monogenic
Twin Pairs Concordant and Discordant
for Schizophrenia

M. Kramer, O. Mendivil Ramos, S. McCarthy,
W.R. McCombie [in collaboration with M. Schatz,
T. Garvin, and H. Fang, CSHL; Fred Gage, The Salk
Institute]

In collaboration with the Gage group, we have se-
quenced the genomes of six identical twin pairs—two
twin pairs discordant for schizophrenia, one twin pair
concordant for schizophrenia, and three control pairs.
Because of the genetic heterogeneity and complexity of
psychiatric disorders such as schizophrenia, we chose
to use the highly identical genomes shared by mono-
genic twins as a filter to subtract background variation
and help elucidate potentially causative mutations.

We were able to produce high-quality libraries with
>34× mean coverage and >92% of bases cov-
ered at 20× or higher depth for each sample. We used
a two-phased analysis to query both shared and dis-
cordant variants between twins who were not seen in
the controls. Our analysis interrogated several aspects
of the genome—namely, SNVs (single-nucleotide
variants), small indels, CNVs (copy number vari-
ants), and structural variants. We used several tool-
sets including GATK, FreeBayes, Scalpel, CNVnator,
and Lumpy.

We first investigated variants discordant between
twin pairs, focusing on rare (<1% MAF) and damag-
ing coding variants. We have identified a novel vari-
ant in the gene ASTN2 in one of the discordant twin
pairs. The variant is a 10-bp frameshift deletion found
only in the affected twin. The astroactin 2 gene is ex-
pressed in the brain and is thought to regulate ASTN1
during neuronal migration. CNVs in this gene have
been previously reported to be associated with autism
and schizophrenia.

We then assessed shared variants between twin
pairs. To reduce the large number of shared variants,
we focused on highly disruptive variants (nonsense,
splice site, and frameshift). We also began as-
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rare/novel variants using in silico enrichment analyses and meta-analysis of networks.

We began with de novo SNV analysis, and we find that the distribution, mutation rates, and functional mutation types follow the same trend as similar published study designs. The preliminary enrichment analyses indicate an overrepresentation of functional de novo variants in the affected probands in gene sets implicated in neuronal architecture (ARC) and increased autism spectrum disorder risk.

We have used several methods to prioritize highly deleterious variants by including information such as gene tolerance to new mutations (RVIS) and variant deleteriousness (PolyPhen and CADD scores). These analyses have highlighted SHANK3 and other genes with de novo mutations in affected individuals as potential candidates.

We are expanding our work to analyze patterns of de novo indels, transmitted variants, and copy number variants.

Targeted Resequencing of 264 DISC1 Interactome Genes in 1543 Samples


In 2015, we improved quality control to improve analysis results. From the previously sequenced 1543 samples, 1446 samples passed these new quality control steps. Among 1446 samples, 575 are case samples consisting of three cohorts: 211 schizophrenia, 195 bipolar, and 169 recurrent major depression disorder. The initial 264 genes were reevaluated based on updated protein–protein interaction databases. The list was trimmed down to 246 genes. We then split the 246 genes into two subsets: 56 direct interactors with DISC1 defined as the DISC1 interactome and an additional 190 genes related to DISC1 expression defined as the DISC1 regulome. Among the SNVs called in the DISC1 interactome and regulome, poor-quality SNVs were excluded and the final data set included 219,648 SNVs.

We characterized the rate and burden of rare (MAF < 1%) and singleton mutations in the DISC1 interactome and regulome that are either disruptive or predicted to have functional effects. We used SKAT for both gene set and gene-wide burden analysis and the Poisson test to assess rates of variants. Based on ANNOVAR annotation, we selected rare and singleton coding variants to test for association in each case cohort and the combined case cohort. We categorized mutations as disruptive mutations (nonsense or splice site; n = 78), nonsynonymous strict damaging (NS strict mutations are those predicted to be damaging by all five algorithms tested; n = 419), and nonsynonymous broad damaging (NS broad mutations are those predicted to be damaging by at least one algorithm; n = 2353). In the DISC1 interactome analysis, we observed that NS broad damaging singleton mutations in DCTN2 showed the strongest association with rMDD (recurrent major depressive disorder), but the finding did not survive multiple testing corrections. In the DISC1 regulome analysis, SCZ (schizophrenia) samples showed a threefold higher rate of rare disruptive mutations than control samples (FWER_within, p = 0.0004; FWER_across, p = 0.0045; FWER is family-wise Type I error rate).

From gene-wide level burden analysis, samples from the rMDD and SCZ cohorts, respectively, showed a significantly higher burden of mutations in TSNAXIP1 with NS strict damaging rare and singleton mutations at a significance level of 0.05 after multiple testing corrections (rMDD FWER_within, p = 0.015; SCZ FWER_within, p = 0.033). There were seven disruptive and damaging variants found in the TSNAXIP1 gene in our data. We found that a nonsense mutation rs146214814 contributed most to the burden of TSNAXIP1 in SCZ samples. Comparing to the frequency of TSNAXIP1 variants in the ExAC (Exome Aggregation Consortium) database (non-Finnish European population), our result suggests a possible SCZ risk variant in an isolated population.

We also tested the burden of DISC1 interactome and regulome on quantitative cognitive ability from control samples. We found that the burden of singleton disruptive variants in the DISC1 interactome showed a significant association with lower cognitive ability at age 11 yr. Also, the burden of singleton NS strict variants was significantly associated with lower cognitive ability adjusted for sex and age. Both disruptive and NS strict variants in the DISC1 interactome showed a significant association with changes in cognitive ability between the ages 11 yr and 70 yr. We are currently finalizing the manuscript for the DISC1 interactome and regulome analysis.

In 2016, we aim to test a broader selection of variants in larger case-control cohorts from Generation Scotland (~10,000 samples) and the National Institute of Mental Health (NIMH) (~7000 samples). We will
use WGS data from the original DISC1 family (in which a majority of affected members carry a balanced translocation between Chr1 and Chr11) to select candidate variants for targeted sequencing of case-control samples. We previously sequenced 49 whole genomes from the DISC1 family. Variant analyses have been performed on those 49 samples to search for novel risk loci, including exonic, intronic, and intragenic regulatory variants. First, we implemented SOLAR for linkage analysis and detected linkage peaks in Chr1, Chr11, and Chr5. The results of single-nucleotide polymorphism (SNP) analysis from the WGS showed the distribution of variants highly shared by affected members was following the pattern of linkage signals. We found that nonsense and splice site mutations in affected members overlapped with de novo mutations in previous autism and schizophrenia studies. We will integrate findings from SNP, indel, and CNV analysis to select candidate genes for targeted sequencing in additional samples. We will also test the effect of selected mutations on genome-wide methylation.

Colorectal Cancer Genomics

E. Antoniou, W.R. McCombie [in collaboration with X. Wang, X. Yu, E. Li, and J. Williams, Stony Brook University]

The incidence and mortality rates of colorectal cancer (CRC) in the United States are higher in African Americans (AAs) as compared with Caucasians (CAs). AAs also develop and are diagnosed with CRC at a younger age compared with CAs. Our hypothesis is that epigenetic changes in the genome of AA patients are driving some of these disparities. It is possible that differences in diet, such as dietary folate and other micronutrients, drive some of these hypothetical epigenetic changes. Because hypomethylation in the promoter regions of tumor suppressor genes and hypomethylated in promoters of oncogenes are known to drive cancer initiation and development, we assessed CpG methylation in AA and CA tumors and paired normal tissues. We also profiled gene expression to identify differential gene regulation between AA and CA tumors.

In late 2014 and early 2015, we did additional sequencing of Reduced Representation Bisulfite Sequencing libraries from six AA and seven CA samples. The data was analyzed using our BStool toolkit (Wang et al. 2015; #8364). When AA tumor methylomes are compared with the paired normal tissues, 1588 hypermethylated and 100 hypomethylated differentially methylated regions (DMRs) were identified. In contrast, only 109 hypermethylated and four hypomethylated DMRs were discovered in the tumors of CA patients. This represents a 14.6-fold and 25-fold, respectively, increase between AA and CA tumors. The gene expression profiling by RNA-sequencing (Seq) revealed 108 down-regulated genes and 34 up-regulated genes in AA versus CA tumors in the same samples. Most of the hypermethylated DMRs (>95%) were mapped to CpG islands, whereas 50% of hypomethylated DMRs were located to CpG islands in AA tumors and 75% of hypomethylated DMRs were located to CpG islands in CA tumors. The highest differentially methylated genes for AA and CA tumors were ranked by statistical significance. Only two genes are common between the top 23 hypermethylated genes in AA and 29 hypermethylated genes in CA samples. Seven microRNAs are also hypermethylated in AA tissues, but only two isoforms of miR-34 are hypermethylated in CA. Gene expression analysis revealed 205 down-regulated and 150 up-regulated genes (FDR [false discovery rate] < 0.05) in AA tumors, whereas only seven genes were up-regulated in CA samples and none were down-regulated. An article is in preparation.

This work was used, in part, to secure a National Institutes of Health (NIH) P20 grant in collaboration with Stony Brook University and State University of New York (SUNY) Downstate. The main goal of this grant is to increase the number of AA and Hispanic tumor samples available for analysis in the next two years. We are planning to sequence the exome and the methylome of these paired tumor/normal samples, and to characterize gene expression using RNA-seq.

PacBio Sequencing of Plant and Complex Cancer Genomes


The utility of next-generation sequencing has been well documented. However, the relatively short reads
generated by most sequencing instruments are inadequate for the de novo assembly of large and complex genomes. The PacBio instrument allows for the sequencing of extremely long fragments, up to ~50 kb. These long fragments provide superior structural information for de novo assembly applications and are important to the understanding of genetic variation.

Previously, we were able to achieve a highly contiguous de novo assembly of the IR64 strain of rice from PacBio sequencing. In-depth analysis has shown superior representation of genes and other features, such as promoters and enhancers, compared with short-read approaches. We also observe a better ability to map RNA-seq results to the long-read assembly, providing vital support to RNA-seq results. Interestingly, the addition of more repeat structures included in the long-read assembly reduces the number of high mapping quality short read alignments. This is a result of more ambiguous mapping introduced by the additional repeats. Genetic annotation and manuscript preparation are under way.

In 2015, a collaboration between Cold Spring Harbor Laboratory and the Ontario Institute for Cancer Research, which began a project to sequence the human breast cancer line SKBR3, was completed. This cell line is an important model for HER2+ breast cancers, one of the most aggressive forms of the disease, which affects one in five cases. The SKBR3 cell line has led to the development of novel approaches to detection of structural variants in long-read data and methods of tracking the mutational history of these variations (Schatz lab). These and other approaches have uncovered 900 intrachromosomal and 300 interchromosomal variants. Additionally, two pieces of software are under development by members of the Schatz lab in direct response to this project: Sniffles, a structural variant detection tool optimized for long-read data, and SplitThreader, a graphical algorithm for exploring and visualizing structural events (both freely available on GitHub at www.GitHub.com). The SKBR3 assembly project and related projects are currently under preparation for publication.

Long-Read Assembly Using Optical Mapping Scaffolds


An associated SKBR3 project involves the use of the BioNano optical mapping system. Over the course of the PacBio SKBR3 sequencing project, it became clear that significant time, effort, and costs are associated with long-read sequencing of complex genomes. Relatively inexpensive optical mapping can provide ultralong molecules (>100 kb) on which to scaffold existing long reads, providing a means to achieve highly contiguous assemblies with less PacBio coverage. A second benefit of optical mapping is the ability to detect long structural variants and validate them on the molecular level, providing significant support for long structural variants discovered in the PacBio-only data.
To evaluate the utility of the BioNano optical mapping system, we generated 125× coverage of SKBR3 using two IrysChips with a molecule N50 of 210 kb. This was then assembled de novo, generating 4105 contigs, an N50 of 0.99 Mb, and covering ~90% of the genome. This assembly was then used to call structural variants. More than 1000 insertions, deletions, and inversions were detected by the BioNano software. Translocation analysis is ongoing. Of the >10 kb structural variants detected by the PacBio-only data, >50% are also found in the BioNano data. This includes a complex nested translocation between Chr8 and Chr17 characterized by nearly 20-fold duplication of the Her2 loci. This nested translocation event was initially discovered in the PacBio-only data and highlights the utility of long-read sequencing.

To assess the extent to which optical mapping data can be used to improve genome assembly, FALCON corrected reads were used to downsample the PacBio data for use in a hybrid assembly approach. The choice to use FALCON corrected reads was made because of the substantial number of computational hours required to correct the initial PacBio data. The use of the precorrected reads allowed for this pilot study to be completed in a timely manner, and new down-sampling experiments will be performed as better tools become available.

The FALCON corrected reads constituted 10× coverage of the genome and were downsampled to 8×, 6×, 4×, and 2× coverage. The corrected reads were then assembled using the CANU assembler, which has options for precorrected PacBio data. Each de novo PacBio assembly was then scaffolded onto the BioNano data. Unsurprisingly, the quality of the hybrid assembly was greatly dependent on the quality of the de novo PacBio assembly. The 2× and 4× PacBio assemblies covered only 10% and 30% of the genome, respectively, and thus yielded poor results. The hybrid scaffold generated with the full set of corrected reads generated an N50 of 5.5 Mb with some contigs spanning nearly whole chromosomes arms; this was on par with the hybrid scaffold generated with the FALCON assembled reads. Six times and 8× downsampled scaffolds generated N50s of 2.3 Mb and 3.6 Mb, respectively, which is similar to the de novo PacBio FALCON assembly N50 of 2.4 Mb. This indicates that as much as 40% less data may be required to generate a highly contiguous assembly when BioNano data is included. Currently methods to optimize the BioNano assembly and structural variant detection are ongoing with an expected completion date by late 2016.

**PacBio Sequencing and Analysis Optimization**


PacBio optimization has been an ongoing project. As a PacBio core facility, we receive DNA samples from all around the world. However, sample quality can vary widely based on target, purification, storage, and shipment conditions. Consequently, although most of these DNA samples pass QC (quality control), some 20-kb insert libraries still result in poor SMRT (single-molecule real-time sequencing) cell yield (<400 million) or short read lengths (<8 kb average polymerase read length). Although acquiring a new DNA sample is an option, there are times when replacing these DNA samples from our users is not possible or when time constraints are prohibitive. To meet the needs of our collaborators and to preserve precious samples, we are researching methods to rescue these libraries.

One potential cause of poor-performing PacBio libraries is single-stranded nicks in the SMRTbell, leading to loss of polymerase processivity. We are currently developing a method for identifying and quantifying the level of single-stranded nicks in PacBio libraries. By virtue of being circular, the SMRTbell molecules in unannealed PacBio libraries have no free end and, thus, the terminal transferase enzyme can be used to add fluorophore-labeled bases to any single-stranded nick in the library. We have developed an approach in which all free ends are labeled in the PacBio library, and a high level of signal is indicative of a large number of breaks and nicks.

In addition to detecting the nick, we have developed a method using *Escherichia coli* ligase to repair poor-performing libraries immediately before instrument loading. We have found that ligase treatment can improve P1 loading efficiency for poor libraries by as much as 50%, and can result in modest gains in both polymerase and subread length with no adverse effect on quality. This process is ongoing and will be included as part of a larger manuscript investigating poor-performing libraries.
Beyond simply repairing poor-performing libraries, we are also interested in the cause of poor library performance. DNA damage that causes loss of polymerase processivity will generate a fragment in which the terminal bases mark where the polymerase stopped. We sought to understand what the expected distribution of terminal k-mers in ideal PacBio libraries is compared with a poor one. To this end, a computational simulation tool, PBlibsim, was implemented in Python to generate simulated PacBio reads. PBlibsim relies on a user-supplied reference genome, as well as user-supplied parameters for the distribution of read length.

In silico reads were used to compare with actual libraries of varying levels of performance from multiple species to further identify and evaluate potential patterns in sites of DNA nicking and associated enrichment of certain regions in poor-performing libraries. The analysis of nicking sites focused on terminal dimers, the pair of nucleotides immediately preceding the nicking site. Distributions of terminal dimers in simulated reads from PBlibsim were constructed and tested to assess significance of positive or negative enrichment in relation to both terminal dimers and absolute dimer count (every possible pair of nucleotides in the genome). Additionally, poor-performing PacBio libraries were bioinformatically compared with in silico data to reveal patterns of terminal dimer enrichment. An enrichment of certain dimers, most notably CT, CG, and GG, is evident in poorly performing libraries. Highly similar patterns were replicated in apple, yeast, duckweed, SKBR3, yeast, suggesting that observed changes in terminal dimer distributions are not unique to any one species. Statistical analysis revealed a high level of statistical significance to differences in terminal dimer distributions, both for overrepresented and underrepresented dimers. In 2016, we are continuing to investigate this topic by expanding the breadth of our bioinformatic pipeline to further elucidate patterns of poor performance.

Sequencing of a Eukaryote Genome and Technology Development on the Oxford Nanopore MinION

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The Oxford Nanopore MinION device is a small DNA sequencer capable of delivering read lengths in excess of 40 kb. This is achieved by passing long DNA fragments through a biological pore imbedded in a membrane within a flow cell. During DNA translocation, a current is passed through the pore and a slight shift in the current profile can be translated into a DNA sequence. Over the course of 2015, we have seen improvements in flow cell performance and reliability, with the most recent flow cell version achieving a 95% accuracy rate in some cases. However, because of the high error rate and limited tools available for such long reads, we found it necessary to develop a novel pipeline to use MiSeq data to error correct the nanopore reads. This pipeline is called nanocorr and is freely available via GitHub at www.GitHub.com. The Celera assembler was used on the corrected yeast nanopore reads to create a highly contiguous assembly resulting in >99.8% consensus identity and an N50 10× greater than an Illumina-only assembly. Using this method we were also able to generate a single contig assembly of a publicly available E. coli nanopore data set. This work was published in Genome Research, October 2015. To date this is the largest genome sequenced and assembled using Oxford Nanopore data.

Parallel to our work with yeast, our group has been a member of the MinION Analysis and Reference Consortium (MARC). This consortium was formed to evaluate and generate reference data in a coordinated, real-world fashion. Six laboratories in the United States and the European Union participated in generating replicate sequencing experiments (four flow cells per laboratory) of E. coli k12. The data from these flow cells was compared and examined in depth to exhaustively evaluate the performance of the MinION. On average, 115 Mb of two-dimensional (2D) bases were generated each with a mean length of 6500 bp and mean quality score of 11.2. The consortium also found that base call accuracy decreases as the experiments progress, but the mean error over an entire experiment is ~12% with deletions slightly dominating over insertions or miscalls. These and other results constitute a snapshot of the MinION performance as of April 2015 and will provide researchers with an invaluable tool for deciding if the MinION will meet their research needs. The consortium goes further in identifying places in which improvements are needed in the MinION chemistry and platform, thereby providing the community a glimpse of changes that might be coming in the future.
Our group continues to be involved in planning and experiments with the MARC consortium. The consortium is currently in the next phase of evaluation experiments and is expanding beyond genomic DNA (gDNA) sequencing into evaluation of the performance of cDNA sequencing. The initial data release from the first-phase data became available in April 2015 and the corresponding publication is available through F1000Research.

As part of our continued evaluation of the MinION device, in 2015 we embarked on two additional projects to optimize the device and to exploit its unique capabilities. The first project aims to reduce the error rate of reads generated by the MinION. Other sequencing approaches reduce the final error by generating a consensus sequence based on many molecules or data from the same molecule sequenced many times. Although the nanopore device does generate a consensus derived from the sequencing of both the forward and reverse strand of a single molecule, these 2D reads do not sufficiently lower the error profile. We are developing an approach in which relatively short molecules (up to ~3 kb) are circularized and amplified using rolling circle amplification (RCA). This creates a concatamer that can be aligned to itself, generating a consensus sequence derived from many copies of the same molecule. A similar approach was used recently with ribosomal RNA (rRNA); however, efficiency is low. Although the efficiency of our approach is also low (<10% of reads are identified as concatamers), we are optimistic that our use of several specific priming steps and unusual polymerases will lead to more efficient library generation. We are currently in the final stages of protocol development and optimization and will begin new sequencing experiments in early 2016. Once an efficient sequencing protocol is developed, we will begin writing algorithms for aligning the concatamer molecules.

The second ongoing project capitalizing on the capabilities of MinION does not focus on its long reads. Rather, this project exploits the rapid speed of library generation (~2 h) and sequencing (<48 h) that can be achieved on this device. Unlike most other sequencing applications, copy number variation (CNV) detection does not rely on high coverage to identify variants. This makes CNV detection well suited to the relatively low yield generated from a single MinION run. Furthermore, CNVs are known to be associated with poor prognosis in several aggressive cancers, including acute myeloid leukemia (AML). Rapidly and inexpensively generating a CNV profile for patients in a point-of-care setting can help dictate the appropriate treatment course to maximize patient survival.

As an initial trial, we began looking for CNVs in the human breast cancer line SKBR3. This line is characterized by many structural events and CNVs, including a >20-fold duplication of the Her2 oncogene. We have tried four separate sequencing runs to optimize the library preparation protocol and determine how well CNVs can be detected. Previous work by the Williams group at Albert Einstein College of Medicine has shown that aneuploidy can be detected with short nanopore reads. However, it was unclear whether smaller CNV events could be detected. Three of the four flow cells generated between ~150,000 and >300,000 reads—enough data to call CNVs. One flow cell generated ~80,000 reads. From the three well-performing flow cells, more than 50% of the reads mapped to the hg19 reference; Ginkgo was used to call CNVs. Several major CNV events, including the Her2 duplication, were identified in each of the samples. Currently, library preparation optimizations are ongoing and we expect to begin testing this method on clinical samples in early 2016.

PUBLICATIONS

PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware   M. Campbell   Y. Koung Lee   J. Mulvaney   M.K. Tello-Ruiz   S. Wei
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D. Ding   S. Kumari   A. Olson   P. Van Buren
A.L. Eveland   C. Liseron-Monfils   M. Regulski   B. Wang
Y. Jiao   Z. Lu   J. Stein   L. Wang

Our lab has two primary goals: (1) research into plant genomics, with a focus on functional and comparative genomics of model plant systems and agriculturally important crop plants; and (2) development of tools, data sources, and resources for the genomics research community. We also contribute actively to three large-scale, multi-institutional, cyber-infrastructure collaboratives designed to serve broad research and educational communities: Gramene, the U.S. National Science Foundation (NSF) CyVerse project, and the U.S. Department of Energy (DOE) Systems Biology Knowledgebase (KBase).

Our lab is a dynamic research group and its composition has continued to evolve, keeping pace with its research objectives. During this period, two of our postdoctoral researchers, Christophe Liseron-Monfils and Christos Noutsos, completed their fellowships and advanced to newer career opportunities, whereas Michael Campbell joined our team as a new postdoctoral researcher. On a similar note, Jurandir Vieira de Magalhaes successfully concluded his collaborative research with the lab as a visiting scientist from Embrapa Maize and Sorghum, Brazil, whereas Dong Ding joined as a new visiting scientist from Huazhong Agricultural University, China.

PLANT GENOME RESEARCH

In the last decade, the sequencing and annotation of complete plant genomes has helped us understand plant function and evolution, as well as how to alter economically important traits. Efforts in many disparate disciplines are required to generate reference genomes. The work starts with laboratory scientists, who generate the raw sequence data. Next, computational biologists and bioinformaticians, such as those in our lab, kick off a series of steps to interpret the raw data. The process of interpretation involves the assembly of raw sequence reads into overlapping segments (“contigs”), which are combined to create a scaffold. This scaffold, in turn, discerns the position, relative order, and orientation of contigs within the chromosomes. The next step is annotation, the discovery and description of genes and other functional elements, and homologies (evolutionary relationships) with other genomes. This information must be faithfully communicated and visualized in Web-based platforms, such as Gramene.

All of these activities are rapidly evolving with the advent of new sequencing technologies, algorithms, and data-handling requirements. For example, high-depth and low-cost sequencing of RNA transcripts is providing a vast stream of new evidence that informs genome annotation; this in turn has spurred the development of new software for modeling and performing genome annotation. Low-cost sequencing has also made it possible to ask whole new classes of questions, moving beyond the generation of single references for individual species and supporting the development of multispecies representation as a “pangenome.” Ongoing projects within the maize, rice, and Arabidopsis research communities are now sequencing hundreds or thousands of genotypic backgrounds, chosen from carefully constructed populations, wild populations, and breeding germplasms in each species. Information about 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 variation not caused by changes in the 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 types of modifications can be studied using new sequencing technologies and analytical methods.
Updating the Maize B73 Reference Genome and Annotation Using Long Sequence Read Technologies

Y. Jiao, B. Wang, M. Regulski, A. Olson, J. Stein, M. Campbell [in collaboration with The Maize Genome Sequencing Consortium; J. Glaubitz, E. Buckler, Cornell University; R. Fulton, R. Wilson, Washington University; K. Guill, M. McMullen, Missouri University, USDA ARS; D. Rank, P. Peluso, T. Clark, Pacific Biosciences; and A. Hastie, BioNano Genomics]

Maize is an important feed and fuel crop, as well as a model system in developmental genetics. A complete and accurate reference genome is imperative for sustained progress in understanding the genetic basis of trait variation and crop improvement in maize. Our laboratory has played a key role in development and stewardship of the maize reference genome since the inception of the maize genome project almost a decade ago. Although the current B73 reference sequence has seen incremental improvements, many gaps and misassemblies remain because of technical limitations in sequence technologies relative to the complexity of the maize genome. To remedy this, we are using PacBio long read sequencing technologies to build the next-generation maize reference genome. A total of ~65X coverage of B73 genome long reads were generated. The N50 of the reads in this data set is nearly 15 kb, with 43.5% of reads longer than 10 kb. To construct a de novo assembly, we have used the PbcR-MHAP correction and assembly pipeline. After optimization, this method yielded an assembly of 3303 contigs with a total length of 2.10 Gb. The N50 and average size of the contigs reached 1 Mb and 634 kb, respectively. To validate the quality of contigs, we aligned them to 28 finished bacterial artificial chromosome (BAC) sequences. Of these, 25 BACs were completely covered by single contigs, demonstrating the ability of contigs to correct and fill gaps in the current reference. Using the maize physical map and a high-density genetic map, we were able to place 96% of contigs and 99% of the sequence into chromosome-level scaffolds. Using the same long read data, we have also tested the Falcon assembly method and achieved a de novo assembly with an N50 of 1.9 Mb, with single contigs able to completely cover 27 of the 28 tested BACs. We are currently working with optical maps to further evaluate these assemblies and to aid with construction of pseudomolecules. We are also developing an efficient maize gene annotation pipeline to support the release of this new version of maize reference genome.

Uncertainties about the complete structure of messenger RNA (mRNA) transcripts, particularly with respect to alternatively spliced isoforms, can be a limiting factor for research in the system. In addition to working on the reference genome sequence, we are using the same single-molecule sequencing technology to investigate the maize transcriptome. For this work, we have sampled full-length cDNAs from six tissues of the maize inbred line B73 that were barcoded, pooled, size-fractionated (<1 kb, 1–2 kb, 2–3 kb, 3–5 kb, 4–6 kb, and 5–10 kb), and sequenced on the PacBio RS II platform with P6-C4 chemistry. We were able to capture 111,151 transcripts representing ~70% of the genes annotated in the current maize RefGenV3 genome assembly. A large proportion of transcripts (57%) are novel. We were able to validate ~90% of the transcripts’ splice-site junctions within high-depth short reads generated from the matched tissues. In addition, we identified a large number of novel long noncoding RNAs (lncRNAs) and fusion transcripts. Our results show that the characterization of the maize B73 transcriptome is far from complete, and that maize gene expression is more complex than previously thought.

Gramene: Comparative Genomic Resource for Plants


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.gra.mene.org, facilitates studies of gene function by combining plant genome and pathway annotation with experimental data and cross-species comparisons. In other words, the data and tools in Gramene enable plant researchers to use knowledge about gene function in one species to predict gene function in other species. This ability should advance our fundamental understanding of plant physiology, including economically important traits such as hybrid vigor, grain development, seed dormancy, flowering time, drought tolerance, and resistance to diseases. Last year, we significantly streamlined the user interface and back-end functions. The
project accomplished several major milestones, culminating in data build 48b (November 2015), our 50th release since the inception of the project (Fig. 1). Improvements to our genome repository include updated assemblies for bread wheat (Triticum aestivum), tomato (Solanum lycopersicum), Medicago truncatula, Oryza meridionalis, and Oryza longistaminata. We also incorporated new genetic variation data (Fig. 2) for S. lycopersicum (from the 100 Tomato Genomes Project), Hordeum vulgare (new POPSEQ marker, variation data from the Leibniz Institute of Plant Genetics and Crop Plant Research [IPK], and new variation identifiers from the transPLANT variation archive), T. aestivum, Oryza glumaepatula, and ethyl methanesulfonate (EMS)-derived point mutant data for Sorghum bicolor, bringing genetic variation for a total of 11 crops. Furthermore, we expanded our Plant Reactome database, which now includes approximately 240 rice pathways (~80% manually curated) and orthology-based pathway projections in 58 species.

Figure 1. Complete reference genomes in Gramene as of data release 49 (January 2016) including 21 monocotyledonous crops and model organisms, 12 dicotyledonous species, one basal angiosperm (Amborellales), and five lower plants (Lycophyta, Chlorophyta, Rhodophyta, and Bryophyta).
For the first time, Gramene processed EMS-mutation data for inclusion in its genome browser. EMS is a mutagenic compound that produces random point mutations at a single nucleotide level. The whole-genome sequencing of 256-phenotyped BTx623 sorghum mutant lines revealed >1.8 million canonical EMS-induced mutations, affecting >95% of genes in the sorghum genome (Jiao et al. 2016). The vast majority (97.5%) of such induced mutations were found to be distinct from natural variations. These variants, similarly to naturally occurring single-nucleotide polymorphisms (SNPs) and structural variants, are displayed in the context of gene annotation and inferred functional consequences that can be assigned to individual accessions within a sampled diversity panel.

The Gramene team develops comparative genomics databases in collaboration with the Ensembl Plants project at the European Bioinformatics Institute (EBI). Perhaps the most significant achievement from this collaboration was the new bread wheat chromosome-scale pseudomolecules assembly using existing International Wheat Genome Sequencing Consortium (IWGSC) chromosome survey sequencing (CSS) contigs and incorporating POPSEQ data from the IPK and processed by the Genome Analysis Centre (TGAC).

Gramene is building integrated data stores from these primary data sources to complement the representational state transfer (REST) application program interfaces (APIs) offered by Ensembl and Reactome. Lightweight node.js web servers provide HTTP access to the integrated data and auxiliary structured annotations stored in MongoDB and Solr document collections. Unified access to these services is provided through http://data.gramene.org, which is built using Swagger middleware.

The Gramene search interface (http://search.gramene.org; Fig. 3) differs from most other search interfaces in that it guides the user to select filters based on gene identifiers, names, and descriptions, as well as structured annotations such as InterPro.

**Figure 2.** Variant image view in Gramene’s genome browser (build 49). Single-nucleotide genetic diversity displayed in the context of functional protein domains.
domains, gene ontology terms, and Plant Reactome pathways. Complex queries can be composed by adding more filters. Search results are presented in multiple ways. A paginated list of matching genes is displayed in which each gene can be dynamically loaded to reveal detailed views that can suggest additional search filters. Search results are also summarized via facet counting, which can rapidly calculate the genomic distribution of gene search results across all hosted genomes. An interactive visualization appears above the search result list.

Having emerged ~15 million years ago (MYA), the *Oryza* genus now includes two cultivated species, Asian rice and African rice, and 21 wild species adapted to a broad range of tropical and subtropical habitats around the world. Genomics research motivated to exploit this diversity for crop improvement is also establishing *Oryza* as a model system for genus-level study of trait evolution, speciation, and domestication. Toward these goals, projects including the NSF-funded *Oryza* Genome Evolution (OGE) Project and the International *Oryza* Map Alignment Project (I-OMAP) have produced high-quality reference assemblies for half of the *Oryza* species. In collaboration with the OGE Project and I-OMAP, Gramene has built a dedicated resource to facilitate comparative and functional genomics research within the *Oryza* genus. The site (oge.gramene.org) features chromosome-level assemblies of 11 genomes—*Oryza sativa* ssp. japonica, *O. sativa* ssp. indica, *Oryza nivara*, *Oryza rufipogon*, *Oryza barthii*, *Oryza glaberrima* (African rice), *O. glumaeapatula*, *O. meridionalis*, *Oryza punctata*, *Oryza brachyantha*, and *Leersia perrieri* (outgroup)—and partial reference assemblies for an additional four *Oryza* species. Structural and functional annotations are presented in genome browsers that promote interspecies navigation across ancestrally conserved regions via whole-genome alignments, phylogenetic gene trees, and gene-level synteny maps. This resource is distinct from the main Gramene site (www.gramene.org), which is committed to supporting community-recognized annotations (e.g., the Rice Annotation Project Database [RAP-DB]) for all its reference genomes. In contrast, the new OGE/I-OMAP database, although still displaying community annotation tracks, features all new annotations derived from methods and evidence that were uniformly applied across each reference genome. The purpose is to enable more meaningful comparisons among gene complements by minimizing confounding artifacts derived from inconsistent annotation.
and screening methods. Resulting annotations were used to build phylogenetic gene trees and define orthologous and paralogous relationships using the Ensembl Compara gene tree method. To compare these genomes, we have applied systematic annotation and phylogenetic analysis of protein-coding genes. Thousands of novel gene families are found at each level of the Oryza species tree. Presence/absence at the gene family level correlates with age of gene family emergence, showing a pattern of decreased stability in newer gene families. Nonsyntenic and novel species-specific genes are not evenly distributed relative to conserved syntenic genes and show clustering in pericentromeric regions. These results are yielding new insights into the taxonomic origin of genes and patterns of duplication, movement, and loss influenced by genome architecture.

PLANT SYSTEMS BIOLOGY
Exploring Arabidopsis Gene Regulatory Networks

Plant microRNAs (miRNAs) play important roles in regulating plant development and stress responses by posttranslationally repressing the expression of their target genes. To identify upstream regulators of miRNA expression, we generated the Arabidopsis miRNA Gene Regulatory Network (ARMIG) using a yeast one-hybrid (Y1H) approach. Using a nearly complete root transcription factor (TF) library, we screened 180 miRNA promoters, their targets, and TFs that are highly connected within the network, and obtained 5376 protein–DNA interactions (PDIs). ARMISs are characterized by highly connected genes as “hubs” in the network. The ZF-HD TFs were identified as hub genes within the miRNA network. An initial survey of six single loss-of-function mutants identified from existing transfer DNA (T-DNA) collections revealed no observable root phenotypes, suggesting that the ZF-HD TFs are functionally redundant. To test this hypothesis, we generated multiple loss-of-function mutants using different approaches, including genetic crosses to a single loss-of-function mutant, generation of artificial micro RNA (amiRNA), and independent repressor lines (Fig. 4). Combining multiple loss-of-function mutants within a single Arabidopsis line, we observed several phenotypes, including altered flower structures and an increased vegetative branching. In addition, we performed transcriptome profiling of ZF-HD TF mutants to characterize differentially expressed genes. The gene-expression profiles and phenotypic properties of the mutants suggested a role for ZF-HD TFs as regulators of developmental transitions. Moreover, the results of this work show that the miRNA GRN can be applied more generally, beyond the root system.

To understand molecular mechanisms of ZF-HD TFs, we use RNA-sequencing (RNA-seq) analysis to reveal gene perturbation between wild-type and loss-of-function mutants, including amiRNA transgenic line, hh23/31/33/34 mutant, and two independent repressor lines, 35s:HB21:SRDX and 35s:HB31:SRDX. In the hh23/31/33/34 mutant, 91% of differentially down-regulated genes are found in the amiRNA line, compared with 48% and 53% of Discovery Environment (DE) genes in the HB31 and HB21 repressor lines, respectively. A similar pattern was observed for DE putative transcription factor genes. This indicates that the ZF-HD TF family has a common and converged biological role, but a distinct functional role in the early flower tissue. To find out the real target of ZF-HD TFs, we are trying to overlay with ChIP-seq data and RNA-seq data. To apply this in crop plants, we are screening the mutant population of maize and sorghum.

The bZIP TFs are another highly connected TF family. One member of the family, GBF2, binds many miRNA and miRNA-target promoters. As with the ZF-HD TFs, single-gene loss-of-function mutants do not have clear phenotypes. We are in the process of generating multiple loss-of-function mutants by performing genetic crosses among the available single-gene mutant lines. Initial screens for homozygous mutants from multiple loci suggest that these mutants may be lethal or have large fitness disadvantages.

Developmental Networks Controlling Inflorescence Architecture in Grasses
A. L. Eveland, S. Kumari, C. Liseron-Monfils, Y. Jiao [in collaboration with D. Jackson, CSHL; and Z. Xin, J. Burke, USDA ARS]

The goal of this work is to integrate genetics and genomics data sets to find molecular networks that influence the morphology (architecture) of grass inflorescences (flowers). Because inflorescences bear the fruits and
grains that we eat, the genetic and regulatory factors that govern their formation are clearly relevant to important agronomic traits such as grain yield and harvesting ability. Our work in maize focuses on 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 established a robust system for investigating the networks that modulate branching, including characterization of precise timing of developmental events and the associated spatiotemporal changes in gene expression. We integrated genome-wide mRNA-seq data to resolve coexpression networks during key stages of maize inflorescence development, and we are currently working to expand these networks by incorporating additional data sets, including genome-wide TF occupancy profiles and cis-regulatory information.

In addition to maize, we have begun work on sorghum, an important emergent bioenergy crop that is also used for human consumption in sub-Saharan Africa. In particular, we are using next-generation sequencing approaches to identify single-nucleotide mutations associated with an increase in seed number. 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 a large-scale mutant study aimed at identifying candidate genes associated with developmental traits in the roots, shoots, and flowers of sorghum.

In the next phase, we will prioritize candidates identified in this stage of the project by overlaying Arabidopsis regulatory network information (see the previous subsection). The resulting hypotheses (e.g., those related to stress responses) can be tested in Arabidopsis and ultimately translated into agronomic systems. Candidate genes that are maize- and/or grass-specific are also of high priority because they
may contribute to the unique morphology of maize inflorescences and/or features shared among other grasses. In addition, we will use comparative genomics approaches, including both cross-species comparisons 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**

**CyVerse (formerly The iPlant Collaborative)**

L. Wang, Z. Lu, K. Chougule, P. Van Buren, D. Ware [This project is in collaboration with CSHL, uses more than 100 staff, and is headquartered at the University of Arizona (PIs: A. Parker, D. Ware, N. Merchant, M. Vaughn, and E. Lyons). Dozens of collaborators are located at more than 20 institutions.]

Our world is changing rapidly. The human population is increasing, whereas arable land and fisheries are decreasing, and food cultivation is being diverted for fuel production. Climate instability and energy sustainability are impacting agricultural and ecological systems, whereas concomitant changes in land-use patterns affect global biodiversity. To successfully address these issues, we need to understand how organisms’ appearance, physiology, and behavior are shaped by the interactions between their genetic makeup and the environment. Although these global challenges are sobering, the efforts to respond productively will lead to exciting science—provided that the computational infrastructure is in place to handle the necessary data sets, analyses, interpretation of results, and dissemination of knowledge. Advances in biological research technology have enabled scientists to amass unprecedented amounts of data, and many researchers find themselves drowning in this sea of data. Foreseeing this major bottleneck in biological research, the NSF established the iPlant Collaborative (http://iplantcollaborative.org) in 2008 to develop cyberinfrastructure for life sciences research and democratize access to U.S. supercomputing capabilities.

Having completed its first 7-year grant, the CyVerse Collaborative has made extensive progress toward meeting these goals. Work in the last year culminated in the development of multiple platforms that are now being used by the research and educational communities. Staff at CSHL contributed directly to some of these cyberinfrastructure platforms or built on existing platforms to provide scientists and educators with ready access to needed software and analysis tools. Within our lab, these platforms include the CyVerse Data Store, Discovery Environment (DE), and Atmosphere. In addition, our lab has successfully built the first federated CyVerse system at CSHL that supports a dozen high-performance computing (HPC) apps running on a local cluster and storage system.

The CyVerse Data Store was designed as a distributed storage system for hosting data on the cloud, providing convenient access. The distributed system allows us to “bring the infrastructure to the data” to enable fast computing, and at the same time, to reduce the bandwidth required to transfer large amounts of next-generation sequencing data. Currently, the CyVerse Data Store consists of two major storage systems with fast internet connections, one at the University of Arizona (UA) and another one at the Texas Advanced Computing Center (TACC). CSHL itself has somewhat limited bandwidth relative to TACC and UA, making our institution an ideal case for validation of the CyVerse model of bringing the infrastructure to the data. Because it makes more sense to use local computing resources with CyVerse prebuilt analysis workflows via the CyVerse AGAVE API than to copy the data to outside clusters (e.g., it takes >10 min to move 1 GB data from CSHL to the TACC clusters), our lab at CSHL decided to and have successfully migrated the important CyVerse platforms at CSHL. The first target is the data store, and a local resource server (WildCat) has been successfully added into the CyVerse storage system, allowing data transfer that is 80 times faster than with outside servers. For the second step, our lab has successfully synchronized the resource server with CyVerse’s AGAVE servers at TACC, which allows CyVerse’s prebuilt workflows to be run locally on CSHL computing servers. The migration of these important platforms provided a proof-of-concept demonstration of the portability of CyVerse Platforms for enrolling more institutes for efficient data management and possibly use of national computing resources provided with good bandwidth.

The DE is the most visible portal for CyVerse 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 CyVerse staff and the user community, or add their own tools to use privately
or share. Although users may not be aware of this, the underlying infrastructure provides access to CyVerse's massive data store at UA and 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, more than 300 tools have been integrated into the DE; these tools enable a broad range of research activities, including genome/transcriptome assembly, annotation, RNA-seq quantitation, variant detection, genome-wide association study (GWAS), and phylogenetics. Members of our lab have played important roles in contributing to workflow design, tool integration, validation, science tutorials, and documentation.

Atmosphere is CyVerse'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 precustomized, ready-to-use software. Users can also create their own applications and environments as VMs and share them with others via Atmosphere. As with the DE, Atmosphere is a gateway to CyVerse's core infrastructure resources, including the high-performance grid computing environment and big-data storage system. Using the Atmosphere platform, we created a VM to be used for image analysis based on high-throughput phenotyping.

The success of genome research depends on our ability to accurately assemble, annotate, and derive meaning from sequence data; however, the extremes of genome size, polyploidy, diversity, and repeat content push the limits on the algorithms, expertise, and computational power currently available to researchers. In response, CyVerse is fostering a community effort to identify best practices and state-of-the-art tools, install them, optimize their performance on the nation's most powerful supercomputers, and make them available as free online resources. Over the last three years, the CyVerse DE has matured to provide a comprehensive set of tools and services for sequence handling, read alignments, RNA-seq profiling, and de novo genome and transcriptome assembly (Fig. 5). To extend these capabilities, we have incorporated 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 provides high-quality, full-fledged annotation pipelines on even the largest plant genomes in a matter of hours. MAKER-P is currently available for use as an Atmosphere image.

A major mission of CyVerse is to promote adoption of the cyberinfrastructure through training workshops and outreach at academic institutions and scientific meetings. In 2015, members of our lab participated as instructors in several “Big Data” workshops focused on transcriptomics and population genomics using the DE and Atmosphere platforms.

**KBase: Department of Energy Systems Biology Knowledgebase**

V. Kumar, S. Kumari, J. Thomason [In collaboration with DOE national laboratories, led by principal investigator A. Arkin, Lawrence Berkeley National Laboratory (LBNL); with co-principal investigators R. Stevens, Argonne National Laboratory (ANL); R. Cottingham, Oak Ridge National Laboratory (ORNL); S. Maslov, Brookhaven National Laboratory (BNL). D. Ware serves as the Plants Science Team Lead for KBase and the chair of the Executive Committee. Other participating investigators include M. Schatz, CSHL; D. Weston, ORNL.]

The KBase (www.kbase.us) has two primary goals. The scientific goal is to produce predictive models, reference data sets, and analytical tools and to show their utility in DOE biological research relating to bioenergy, carbon cycle, and the study of subsurface microbial communities. The operational goal is to create the integrated software and hardware infrastructures needed to support the creation, maintenance, and use of predictive models and methods for the study of microbes, microbial communities, and plants.

KBase’s computational infrastructure is supported by a distributed, high-performance, cloud-based system that includes >3 petabytes of storage, more than 12,000 cores for data processing, and 90 Gbit/sec bandwidth over DOE’s ESnet. The KBase data model supports more than 900 data types, including sequence reads, assemblies, genomes, annotations, expression data, quantitative phenotype data, and metagenomic profiles. The KBase data repository has integrated various data sets from public resources for thousands of microbes, hundreds of microbial communities, and tens of plants.

In addition, KBase supports more than 1000 analysis and access functions grouped into more than 30 integrated service modules, including sequence assembly,
variation analysis, genome annotation, expression analysis, and various types of modeling (e.g., metabolic, regulatory, and flux balance analysis [FBA]). To capture complex, multistep analyses, users can build persistent and provenance-based workflows that can capture complex, multistep analyses and mobilize rich annotations, visualization widgets, reusable workflows, and custom scripts. This capability empowers users to create science that is transparent, reproducible, and reusable.

In February 2015, the team released the first production version of KBase. The next phase started with a focus on (1) stabilizing and extending the existing system with new functionality as use base expands, (2) streamlining the system architecture to enable users to add new tools and data types and scalably execute complex analyses, and (3) driving development through strategic collaboration with targeted community members to enable new science for next-generation systems. During 2015, the plants team created a variety of analytical workflows that integrate gene expression profiles and metabolic networks, largely supported by the RNA-seq pipeline currently being developed by the plants team. The present version of RNA-seq service (RNA-seq v1.0) is essentially an implementation of the RNA-seq Tuxedo protocol for prokaryotes and eukaryotes. This provides an integrated computational workflow for RNA-seq-based gene expression analysis for microbes and flagship plant genomes. This service aligns the RNA-seq reads, assigns aligned reads to genes, and performs differential gene expression analysis to detect differential transcript abundances among tissues, developmental stages, genetic backgrounds, and environmental conditions.

The current RNA-seq pipeline is based on Tuxedo protocol and will be further extended, as well as hardened, in the near future. Ultimately, this service will support a wide range of transcriptome data analysis needs such as gene discovery/annotation, gene fusion detection, allele-specific expression, and de novo transcriptome assembly.
Also, the plants team has continued to improve on the construction and curation of metabolic models of specific microbe, fungus, and plant genomes and their interactions, to understand plant microbial and mycorrhizal interactions. This has allowed us to generate new hypotheses for metabolic reconstruction and flux balance analyses simulations using the expression matrix to generate the set of reactions active in biomass production.

The plants team also laid the foundation for the homology services, currently in development, by implementing the BLAST-based genomic search utility. The BLAST tool was designed to support the different flavors, such as blastp/n/x and tblastn/x, and supports graphical alignment of the search results.

Another focus within the project is the creation of a library of web interface components (widgets) for rapid development of reusable visualizations that can be dropped into disparate interfaces to present biological data with new insights. Such visualizations help scientists interpret complex data sets. Building on standard web libraries, such as jQuery, Bootstrap, and D3, has allowed the creation of a flexible and very familiar widget standard. This standard provides dynamic visualizations that can immediately update as the underlying data changes, as well as interactive visualizations that allow graphs to operate as user interfaces such that image manipulations translate into actual data modifications in real time.

Figure 6. Gene expression profiling in KBase. KBase provides a variety of plant resources and analytical services that are integrated with gene expression profiles, regulatory interactions, and metabolic networks. For example, users can run RNA-seq pipeline and tools for downstream analysis to quantify expression from the RNA-seq reads, perform transcriptome assembly, gene annotation and identify differential expression between tissues, developmental stages, environmental conditions and genetic backgrounds. Note that Network Inference and Reconstruction (dotted box) is a work in progress.
PUBLICATIONS


In Press

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 timescales 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.

Justin Kinney completed his Ph.D. 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 is devoted to addressing the theoretical and computational problems relevant to the analysis of Sort-Seq data.

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 representing 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.

Adam Siepel and colleagues address various problems in genomics and evolution using tools and techniques from computer science and statistics. Over the years, Siepel’s research has touched on diverse topics, including the identification of recombinant strains of HIV, the discovery of unannotated human genes, the characterization of conserved regulatory elements in mammalian genomes, and the estimation of the times in early human history when major population groups first diverged. A general theme in the group’s work is the development of precise mathematical models for the complex processes by which genomes evolve over time, and the use of these models both to peer into the past and to address questions of practical importance for human health. Recently, they have increasingly concentrated on research at the interface of population genomics and phylogenetics, with a particular focus on humans and the great apes. They also have an active research program in computational modeling and analysis of transcriptional regulation in mammals and Drosophila, in close collaboration with Professor John Lis at Cornell University.

These investigators are all part of the Simons Center for Quantitative Biology (SCQB), CSHL’s home for mathematical, computational, and theoretical research in biology. The central idea behind the SCQB is to place researchers trained in mathematics, physics, computer science, and other quantitative fields on the front lines in biology, working shoulder to shoulder with experimentalists. In addition to collaborative work, SCQB researchers pursue independent research in algorithms, machine learning, statistical genetics, molecular evolution, and other areas. The ultimate goal of the Center is to promote the use of quantitative methods to enable groundbreaking research across a wide variety of biological domains, including human genetics, cancer, plant biology, and neuroscience. The SCQB was created in 2008 with a major donation from the Simons Foundation, as well as gifts from the Starr Foundation and Lavinia and Landon Clay. Adam Siepel was recruited to serve as Chair of the SCQB in 2014.
Fueled by data generated from recent technological developments in DNA sequencing, the Atwal 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. A long-running thread in our work is the analysis of cancer genomes from an evolutionary perspective to determine how this informs on clinical outcome and therapy.

In our lab, we have been following up on the investigation of ectopic expression of germline genes, transcribing genetic loci exclusively expressed in testes/ovaries in various tumors. Previous work in our lab has established the pleiotropic and ancient role of the TP53 pathway in germline development. Preliminary results have also identified ectopic expression of germline piwi genes in samples of glioblastoma multiforme extracted from The Cancer Genome Atlas. However, the landscape of ectopically expressed germline genes is unknown and their functional impact on cancer development remains elusive. We began an integrative study that sought to determine the ectopic expression of germline transcripts in glioblastoma multiforme and breast cancer, elucidating the predictive value of germline molecular programs as diagnostic markers and immunogenic therapeutic targets. Taha Merghoub and Jedd Wolchok at Memorial Sloan Kettering Cancer Center (MSKCC) are new collaborators on this project.

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 haplotype 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 \textit{LIF}, \textit{Mdm2}, \textit{Mdm4}, and \textit{Hausp} genes, each of which lies in the p53 molecular network, were found to be enriched in women undergoing in vitro fertilization (IVF) treatment. Moreover, some of these SNPs have been shown to be associated with estrogen-driven cancer risk, highlighting the pleiotropic character of genetic variants in the p53 pathway. We have also sought 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.

A major and recent research focus of our lab is the investigation of cancer evolution using sequencing data derived from single-cell genomics. Although the timescales vary by many orders of magnitude, the mathematical tools of population genetics, originally devised to model molecular evolution over millions of years, can be repurposed to understand single-cell evolution of tumors in the lifetime of an individual. In particular, we have been addressing the ubiquitous problem of how many cells and how much read coverage do we need before the inferred cell phylogeny accurately reflects the evolutionary history of the tumor. 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.

Robert Aboukhalil, a graduate student from the Watson School, has continued to make progress developing software to analyze copy number data obtained from single tumor cells and protocols for optimal single-cell sequencing. In early 2015, Mickey Atwal developed and taught a new graduate course at the Watson School, Quantitative Biology II, to address the needs of advanced computational students. Specifically, the syllabus was designed to dive more deeply into some of the more advanced machine learning methods, such as Markov chain Monte Carlo (MCMC) and variational approaches, that are currently being developed and deployed in the analysis of biological data, ranging from genomics to neuroscience.
Ectopic Germline Gene Expression in Cancer

This work was done in collaboration with Robert Darnell (Rockefeller University); Astrid Haase (CSHL), and Jedd Wolchok (MSKCC).

We initiated an integrative study that sought to determine the ectopic expression of germline transcripts in glioblastoma multiforme and to elucidate the predictive value of germline molecular programs as diagnostic markers and therapeutic targets.

To determine the genome-wide landscape of restricted germline expression, we developed an empirical Bayesian computational approach to analyze normal human soma and germline tissues represented across 1236 heterogeneous expression data sets. Germline tissues were found to express a significantly large, but tightly knit, network of genes ($n = 1294$, $q$-value = $10^{-2}$) that we inferred to be tissue-restricted, and the functionality of these genes is enriched in molecular pathway processes such as piwi-interacting RNA (piRNA) metabolism ($p = 10^{-6}$) and cell-cycle processes ($p = 10^{-36}$). By analyzing RNA-Seq data from The Cancer Genome Atlas, we identified 385 high-confidence germline genes ectopically expressed in glioblastoma multiforme samples, serving as a potential source of neoantigens. Analyses of clinical survival data revealed a stratification of patients with high and low numbers of germline genes transcripts, demonstrating that the germline genetic program can inform on clinical outcome.

To elucidate the role of the germline piRNA processing pathway in cancer, our collaborators purified ectopic piwi complexes in cultured cancer cells and investigated the identity of associated nucleic acids. Next-generation sequencing revealed that the piwi proteins are not loaded with piRNAs, suggesting a piRNA-independent mechanism for their reactivation. Current efforts are geared toward investigating the association of ectopic germline expression and patient response to immune checkpoint blockade therapy.

Web Platform for Single-Cell Genomics

R. Aboukhalil [in collaboration with the Schatz and Wigler labs, CSHL]

Single-cell sequencing is emerging as a critical technology for understanding the biology of cancer, neurons, and other complex systems. Here, we introduce Ginkgo, a Web platform for the interactive analysis and quality assessment of single-cell copy number alterations. Ginkgo fully automates the process of binning, normalizing, and segmenting mapped reads to infer copy number profiles of individual cells, as well as constructing phylogenetic trees of how those cells are related. We validate Ginkgo by reproducing the results of five major single-cell studies and discuss how it addresses the wide array of biases that affect single-cell analysis. We also examine the data characteristics of three commonly used single-cell amplification techniques, MDA, MALBAC, and DOP-PCR/WGA4, through comparative analysis of nine different single-cell data sets. We conclude that DOP-PCR provides the most uniform amplification, whereas MDA introduces substantial biases into the analysis. Furthermore, given the same level of coverage, our results indicate that data prepared using DOP-PCR can reliably call copy number variations (CNVs) at higher resolution than data prepared using either MALBAC or MDA. Ginkgo is freely available at http://qb.cshl.edu/ginkgo. The Web implementation was spearheaded by Robert Aboukhalil.

Modeling tasiRNA Regulation of Auxin-Response Factors in Moss

This work was done in collaboration with the Timmermans lab (CSHL).

Analysis of Physcomitrella patens mutants perturbed in trans-acting small interfering RNA (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.
Learning Quantitative Sequence–Function Relationships from Massively Parallel Experiments

This work was done in collaboration with Justin Kinney (CSHL).

A fundamental aspect of biological information processing is the ubiquity of sequence–function relationships—functions that map the sequence of DNA, RNA, or protein to a biochemically relevant activity. Most sequence–function relationships in biology are quantitative, but only recently have experimental techniques for effectively measuring these relationships been developed. The advent of such “massively parallel” experiments presents an exciting opportunity for the concepts and methods of statistical physics to inform the study of biological systems. After reviewing these recent experimental advances, we focus on the problem of how to infer parametric models of sequence–function relationships from the data produced by these experiments. Specifically, we retrace and extend recent theoretical work showing that inference based on mutual information, not the standard likelihood-based approach, is often necessary for accurately learning the parameters of these models. Closely connected with this result is the emergence of “diffeomorphic modes”—directions in parameter space that are far less constrained by data than likelihood-based inference would suggest. Analogous to Goldstone modes in physics, diffeomorphic modes arise from an arbitrarily broken symmetry of the inference problem. An analytically tractable model of a massively parallel experiment is then described, providing an explicit demonstration of these fundamental aspects of statistical inference. This paper concludes with an outlook on the theoretical and computational challenges currently facing studies of quantitative sequence–function relationships.

PUBLICATIONS


In Press

DE NOVO MUTATION IN AUTISM

I. Iossifov  A. Munoz Jimenez

The Iossifov lab studies the genetics of common diseases in humans, using two main tools—next-generation sequencing and molecular networks, representing functional relationships among genetic loci—that, 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 the development of new methods (for sequence analysis and building and using molecular networks) and their application to specific biomedical problems.

Low Load for Disruptive Mutations in Autism Genes and Their Biased Transmission

We previously computed that genes with de novo (DN) likely gene-disruptive (LGD) mutations in children with autism spectrum disorders (ASDs) have high vulnerability: Disruptive mutations in many of these genes, the vulnerable autism genes, will have a high likelihood of resulting in ASD. Because individuals with ASD have lower fecundity, such mutations in autism genes would be under strong negative selection pressure. An immediate prediction is that these genes will have a lower LGD load than typical genes in the human gene pool. We confirm this hypothesis in an explicit test by measuring the load of disruptive mutations in whole-exome sequence databases from two cohorts. We use information about mutational load to show that individuals with lower and higher intelligence quotients (IQs) can be distinguished by the mutational load in their respective gene targets, as well as to help prioritize gene targets by their likelihood of being autism genes. Moreover, we show that transmission of rare disruptions in genes with a lower LGD load occurs more often in affected offspring; we show transmission originates most often from the mother, and transmission of such variants is seen more often in offspring with lower IQ. A surprising proportion of transmission of these rare events comes from genes expressed in the embryonic brain that show sharply reduced expression shortly after birth (Iossifov et al. 2015).

Ongoing Projects

Whole-genome sequencing of Simons Simplex Collection (SSC). Whole-exome sequencing (WES) will not exhaust the information available in the SSC; more can be uncovered by whole-genome sequencing (WGS). As with WES, the main goal of WGS is gene target discovery in simplex families through the identification of disruptive DN events. Certainly, all the data that one can derive from exome capture can also be extracted from whole-genome data, hopefully with more even coverage of the exons. By that alone, we anticipate extracting on the order of 30% more events. Additionally, we will observe DN events in promoter regions, evolutionarily conserved regions near target genes, and noncoding genes like micro-RNAs and long noncoding RNAs. These genomic regions are not targeted by WES, yet they may add to the discovery of recurrent targets and to the list of causative mechanisms for genetic disruption. Although the interpretation of any individual event is likely difficult, we can nevertheless calculate a differential signal between affected and unaffected siblings for various regions of suspected functionality. Finally, WGS will certainly facilitate the discovery of small copy number changes and copy number–neutral rearrangements.

PUBLICATIONS


MEASURING AND MODELING QUANTITATIVE SEQUENCE–FUNCTION RELATIONSHIPS

J.B. Kinney  R. Adams  T. Forcier

During my graduate and postdoctoral training, my colleagues and I developed an experimental method, called “Sort-Seq,” that is capable of measuring the transcriptional activities of hundreds of thousands of variant *Escherichia coli* promoters in a single experiment. Through a quantitative analysis of the large data sets that Sort-Seq produces, it is possible to quantitatively characterize the biophysical mechanisms of transcriptional regulation that operate at a specific promoter of interest—that is, to identify transcription factor binding sites, to measure protein–DNA and protein–protein interaction energies, and so on. I believe that Sort-Seq represents just one example of how ultrahigh-throughput DNA sequencing can be used to identify precise quantitative models of complex biophysical/biochemical systems. To realize this vision, my laboratory is pursuing an integrated combination of theory, computation, and experiment. On the experimental end, we continue to develop deep-sequencing-based assays for investigating biochemical systems. We are also developing computational tools for analyzing data that these experiments produce. Our theory work is aimed at addressing unsolved problems in biophysics and machine learning that are highlighted by the availability of Sort-Seq and other massively parallel data sets.

Measuring the Sequence-Affinity Landscape of Antibodies with Massively Parallel Titration Curves

Despite the central role that antibodies play in the adaptive immune system and in biotechnology, surprisingly little is known about how antibody-antigen-binding affinity depends on antibody sequence. For instance, there is virtually no understanding of how many different antibody sequences can bind an antigen of interest with a given affinity, how this “density of states” varies with binding affinity, how “glassy” this sequence-affinity landscape is, etc.

To make progress on these problems, Rhys Adams (Ecole Normale Supérieure [ENS], Paris/CSHL), Aleksandra Walczak (ENS, Paris), Thierry Mora (ENS, Paris), and I have developed a new experimental technique called “Tite-Seq.” This assay is described in our recent preprint. Tite-Seq combines yeast display and fluorescence-activated cell sorting (FACS) in a way that allows the affinity of thousands of variant antibodies to be measured in a single experiment. In contrast to existing deep mutational scanning assays, which measure binding at only a single ligand concentration, Tite-Seq provides full titration curves. Having these titration curves eliminates the confounding factors present in deep mutational scanning assays, such as the sequence dependence of antibody expression and stability. We anticipate that Tite-Seq will, in the near future, provide unprecedented insight into the quantitative structure of antibody-antigen sequence-function relationships.

Learning Quantitative Sequence-Function Relationships from Massively Parallel Data

In contrast to genome-wide experiments such as ChIP-Seq, Sort-Seq and other massively parallel assays provide large quantities of data probing the inner workings of very specific biochemical systems. Quantitative modeling of the sequence-function relationships probed by massively parallel assays, therefore, has the potential to provide precise mechanistic insight into how such systems operate.

Standard methods in statistical inference, however, are not well suited for inferring quantitative models from massively parallel data. The reason for this is that standard inference methods are based on a quantity called “likelihood.” To compute likelihood, one has to make strong assumptions about the quantitative form of experimental noise. The noise present in massively parallel experiments, however, is virtually impossible to precisely characterize a priori because it varies from experiment to experiment in unpredictable ways.

A recent paper (Kinney and Atwal, *Neural Comput* 26: 637 [2014]) and a soon to be published paper
describe a resolution to this problem. In these works, Gurinder Atwal (CSHL) and I show that using a quantity from information theory called “mutual information” in place of likelihood obviates the need to assume a specific model of experiment in these types of inference problems. We further introduce the concept of “diffeomorphic modes”—directions in parameter space that mutual information cannot pin down. The existence of diffeomorphic modes reveals a basic fact about statistical inference that had previously remained unrecognized—namely, that different model parameters can respond to data in qualitatively different ways. This finding is applicable to a wide range of statistical regression problems, including many “big data” problems in biology.

**Field Theoretic Methods for Estimating Low-Dimensional Probability Densities**

As with many efforts to understand experimental data, analyzing Sort-Seq data requires the accurate estimation of continuous probability distributions (also known as probability densities). Despite the ubiquity and seeming simplicity of this problem, the best way to estimate a continuous probability distribution from data remains unresolved, even in one dimension. I believe, however, that field theory methods from theoretical physics can ultimately provide a definitive practical solution to this important statistical problem.

Field theory methods for estimating probability densities date back to the mid-1990s. These methods are computationally intensive, however, and, until recently, no practical implementation of these methods was available. I (Kinney, *Phys Rev E* 90: 011301 [2014]) showed that field-theory-based density estimates could be computed rapidly and deterministically using an efficient homotopy algorithm. This paper also shows, for the first time, that such density estimation methods can perform favorably relative to more standard density estimation methods.

In a more recent paper, I showed that the boundary conditions that had been placed on estimated densities in previous field-theoretic work (including in Kinney, *Phys Rev E* 90: 011301 [2014]) are actually unnecessary. Removing these boundary conditions has an important consequence: It causes the well-known “maximum entropy” approach to density estimation to be recovered in the infinite smoothness limit of the field theoretic approach. This unification of field theory and maximum entropy methods for density estimation suggests a new way of testing the hypothesis that one’s data comes from a probability distribution having a specified functional form.

By establishing the ability to rapidly compute field theoretic density estimates and by showing how to remove prior assumptions about the boundary behavior of these densities, my work provides a solid foundation on which to build a practical solution to the density estimation problem in low dimensions. In the near future, I hope to show how such density estimation methods can be used to accurately estimate mutual information and other important statistical quantities. I also hope to provide easy-to-use software that makes these statistical methods available for general use.

**Modeling Multiparticle Complexes in Stochastic Chemical Systems**

My work on transcriptional regulation requires building quantitative biophysical models of molecular interactions. Currently, the only way to mathematically define models of such interactions is to explicitly list all the possible states of a system. This task, however, becomes exponentially more difficult as system size increases. I believe this exponential explosion in difficulty reflects a fundamental deficiency of the mathematical tools currently available for studying stochastic chemical systems.

To address this problem, Muir Morrison (Caltech) and I developed a mathematical formalism that represents stochastic chemical systems of multiparticle complexes in terms of component particles, interaction energies, and assembly rules. This mathematical formalism also has a diagrammatic representation that greatly aids in its use. In a bioRxiv preprint, we present this formalism and show how it can dramatically simplify the description of both equilibrium and non-equilibrium stochastic chemical systems. Our hope is that these 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**


In Press


IN SILICO CANCER GENOMICS

A. Krasnitz  R. Layne  J. Song  V. Zhygulin

Research in our group is focused on in silico cancer genomics. In the last several years there has been explosive growth in the volume, as well as quality and detail, of cancer-related genomic data available for analysis. This development came about through rapid accumulation of public data sets generated by individual laboratories worldwide, through large-scale cancer genome projects such as the Cancer Genome Atlas (TCGA), and with the advent of new experimental methodologies, especially next-generation sequencing and single-cell genomics. We see our goal in 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, and elucidating the clonal structure of tumors and its relation to progression, invasion, metastasis, and response to treatment. All our work is performed in close coordination with experimental studies performed by the Wigler, Hicks (presently at the University of Southern California), Spector, and Stillman laboratories at CSHL.

Computational Pipeline for Single-Cell Genomics

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; and 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 this data form for robust reconstruction of cancer cell phylogenies is a challenging computational problem calling for new and robust informatic and statistical tools.

We responded to this challenge by developing a computational pipeline for single-cell genomics. The pipeline comprises two major modules, one for deriving integer-valued copy number profiles of individual cells and one for establishing genealogical relations among the cells in a sample and identification of clones. The input into the pipeline consists of cell-specific sets of sequencing reads. These are first aligned with the genome. The sequencing read density as a function of genomic position is then used to derive integer-valued DNA copy number profiles for each cell. Before any further processing these profiles are examined for evidence of extensive DNA damage or degradation, and profiles derived from damaged DNA are filtered out. Next, each of the remaining profiles is reduced to a set of copy number change points, in which, for each change point, the genomic interval of its likely location and the sign of the change are specified.

From this point on we combine change-point reduced copy number profiles of the cells constituting the sample with the ultimate goal of reconstructing their phylogeny. To this end, we first derive a minimal set of features to account for all the change points observed in the sample and construct a table to indicate, for each cell genome, the presence or absence of each feature. Pairwise similarity of cell genomes is quantified based on the number of features shared by the pair. We then examine the resultant similarity matrix for the presence of clones (i.e., groups of cells whose unusually high number of shared features can only be explained by their descent from a recent common ancestor). Such clones may in turn contain subclones of even more closely related cells.

Single-cell data, as interpreted by the pipeline, must be presented to cancer biologists and clinicians
in a comprehensible way in order to have an impact on clinical outcome. In practice, this means that the data should be viewable in an organized way, with the viewer retaining the ability to change the organization. The end user must be able to see and navigate the phylogenetic organization. Moreover, the data and their interpretation must be viewed in the context of other parameters, such as anatomical sites, along with their pathological assessment. Such examination of the data in aggregate offers the best chance to reveal the critical properties of the sample relevant to clinical assessment. An integrated user interface, capable of handling specialized forms of data that arise in single-cell research, is therefore essential. With this necessity in mind, we have created the interactive single-cell viewer (ISCV). The viewer displays multiple single-cell genomic profiles in the chromosomal order, with colors encoding the sign and magnitude of copy number aberrations. The cells on display are ordered as leaves on a tree, reflecting the structure of the population being sampled. In particular, clonal and subclonal identities of the cells are indicated in the appropriate tracks. Other tracks provide information on the cell ploidy, the quality of the cell genomic data and, importantly, the anatomic origin of the cell. Elements of the data can be examined in greater detail by a combination of selection and zoom-in. For example, any number of cells can be selected and the corresponding copy number profiles plotted in a separate interface. A link to the UCSC genome browser is available for any genomic region displayed by the viewer to help put the findings in a broader genomic context.

Recently, we used the pipeline to reconstruct the cell population structure in samples from 10 sets of prostate biopsies, spanning a range of Gleason scores from benign to 9. Some of the biopsies were performed diagnostically, in response to elevated PSA (prostate-specific antigen), whereas others were performed post-surgery. Between 100 and 800 single-cell genomes were acquired by low-coverage sequencing from each prostate. There are several key observations from our analysis of tumor cell genealogies. The first is that cells in higher-grade regions display a well-defined clonal identity and, within each clone, share a substantial number of genomic features. The second observation is that these clones mostly are formed by cells that share an anatomic location. At the same time, we do occasionally find evidence for clones spreading beyond their anatomic origins. Third, copy number profiles of cells in clonal populations grow progressively more complex with the Gleason scores and the populations themselves grow more numerous in the prostatic tissue. Finally, and importantly, we observe that our measures of clonality and complexity are better correlated with the final Gleason score assessment following radical prostatectomy than with the scores assessed from diagnostic biopsy. These findings argue for potential utility of single-cell genomic analysis to complement conventional pathology.

**PanCanAtlas: Stemness and Immune Response across Multiple Tumor Types**

TCGA is a massive, comprehensive data repository for cancer research. More than 30 cancer types are currently represented in the atlas, with hundreds of patient cases per type. For each tumor somatic mutation, DNA copy number variation, DNA methylation, mRNA, and micro-RNA profiles are available, along with clinical annotation for the patient. By combining multiple data sets, TCGA consortium generated integrated molecular portraits of a number of cancer types. PanCanAtlas is a successor project to TCGA, seeking to take data integration one step further and across multiple cancer types. Our group has been part of this effort since June 2015, with two focal areas of interest. The first of these is concerned with quantifying the presence of stem-cell-like cell populations in tumors. In particular, loss of differentiation has long been recognized as a key feature of cancer pathology. We hypothesize that, on a cellular level, this loss of differentiation is accompanied by acquisition of stem-cell-like properties. In an exploratory study, we find that genes associated with loss of differentiation in multiple cancers frequently occur in published transcriptional signatures of embryonic stem cells. In addition, we are interested in pancancer characterization of immune response to tumors, with a particular focus on the role of cancer testis antigens (CTAs)—that is, products of genes that are exclusively expressed in testes of healthy male individuals, where they are protected by immune privilege from exposure to the immune system. Expression of these genes in tumors should therefore elicit an immune response, in particular, by cytotoxic T cells. Such a response is an end result of a multistep process, involving mRNA expression and translation into proteins, followed by
proteasome cleavage, antigen presentation, and recognition by the adaptive immune system. Our ongoing study aims at taking these multiple steps into account to quantify potential immune response to CTA in tumors documented by TCGA.

PUBLICATIONS
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 and protocol development, data analysis, and genetic theory, with a focus on sporadic human diseases such as autism, congenital heart disease, and cancer.

**SMASH Sequencing**

Measuring copy number is accomplished by either array hybridization or standard next-generation sequencing (NGS). Given the exponential improvements in NGS, long-term trends favor the adoption of sequencing technologies. However, much of the gains in throughput observed in NGS derive from longer read lengths. These longer reads improve mapping and coverage, but they do not increase the number of independent maps in a sequencing library. Typically, 35 to 40 bases are sufficient to uniquely map a fragment. Reads that extend 500 base pairs (bp) have considerably more information; however, all those extra bases do little to improve copy number resolution.

Together with Zihua Wang, Peter Andrews, and Michael Wigler, we devised a method to pack multiple short fragments into a single sequencing read and then an informatics method that unpacks the short maps, converting the results into read density and copy number measurements. We call the method SMASH sequencing. To pack multiple maps into a single read, we first fragment the DNA by mechanical and/or enzymatic methods to a mean size of 40 bp, ligate the fragments together, and then adapt for sequencing. Computationally, the reads are analyzed for all the longest possible sequences that map uniquely to the reference genome. These are called maximal unique matches or MUMs. We optimized a heuristic for selecting MUMs from each read and, from that point, applied standard methods for converting map density into copy number profiles.

We tested the method in its two primary use cases: identifying copy number variations (CNVs) in a family and identifying CNVs in a cancer cell line. In both applications, SMASH performed equally to standard sequencing approaches on a map-for-map basis. With SMASH, we obtain about four maps per read such that at present read lengths, SMASH offers a savings of 75% off sequencing costs for copy number analysis.

**Sensitive Detection**

Current Illumina sequencing instruments generate high-throughput, high-quality sequence data with error rates of ~1 per 100 bp read. For many applications, such as determining heterozygous positions in a genome, this is a sufficient level of precision—1% will not introduce a significant error in a measurement of 50 per 100. However, there are applications for which this level of error is unacceptable—in particular, those cases in which we wish to measure a variant that occurs at a rate of <1 per 100. These include important applications like measuring residual disease in cancer, identifying low-frequency subpopulations in a tumor, and determining rates of somatic variation.

To counteract the errors introduced by sequencing and polymerase chain reaction (PCR), together with Michael Wigler and Zihua Wang, we developed a protocol that adds a unique sequence identifier (or “varietal tag”) to the initial template molecule. Because sequence error is sparse and independent of the template molecule, reads with the same varietal tag are unlikely to have the same sequencer errors. This provides a degree of resolution up to PCR error. To counteract PCR error, we introduce rounds of linear amplification and an optional second tag. Because PCR errors are unlikely to occur on distinct lineages, the second tag can be used to ensure that the read data samples independent lineages. The residual differences, then, account for either true variation or template damage. We estimate that this method offers a resolution of between 1 in $10^7$ and 1 in $10^6$ depending on the sequence context.
Genomic Rearrangements

Together with Peter Andrews, Michael Wigler, and Ivan Iossifov, we have developed an algorithm called MUMdex for identifying large genomic rearrangements—deletions, insertions, translocations, and inversions—by cataloging discontinuities in the mapping of reads against the reference genome. Taking advantage of memory-intensive computational methods, we are able to rapidly identify all MUMs between a read and a reference genome. This provides a scaffold for cataloging and indexing all of the sequence data such that we can quickly identify discontinuities: places where placing the reads on the scaffold bends or breaks the reference genome. By looking across all samples, we can identify common events, spurious rearrangements, 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. To analyze large data sets requires an efficient and robust data format and processing algorithms, which we are currently deploying on both the 10,000 SSC exomes and a smaller whole-genome sequence data set.

Mutational Sequencing

Despite vast improvements in DNA sequencing, many problems of interpretation arise when trying to count or assemble molecules (templates) that are largely identical. In a recent paper, we show that by randomly mutating DNA templates before amplification, many of these problems are resolved. We can accurately count the number of templates by counting the number of unique patterns. By introducing distinctive patterns onto otherwise identical spans, we enhance our ability to correctly assemble sequences. This idea has applications in RNA expression analysis, haplotype phasing, copy number determination, and genome assembly. Mutational sequencing (muSeq) solves counting problems and effectively generates long reads from short read sequence data.

We have developed a new bench protocol to generate random, uncorrelated mutations at a tunable rate. By modifying a standard bisulfite sequencing protocol, we are able to randomly deaminate 50% of all unmethylated cytosines. In the context of a sequence read, this mutation converts some fixed proportion of “C” to “T,” a conversion that is inherited in every copy of the template.

We have also developed algorithms for counting and assembling mutational patterns in the noisy context of real sequence data. In developing a method to cluster reads with the same mutation pattern, we designed a new, general purpose clustering algorithm that we call “transitive propagation.” Modeled after affinity propagation, our method balances pairwise measures of “sameness” and “difference” while restricting to solutions that preserve the “transitivity of sameness”: If \( a \) is the same as \( b \) and \( b \) is the same as \( c \), then \( a \) is the same as \( c \). This algorithm solves our immediate problem of clustering reads by mutation pattern in the face of sequence and PCR error. However, it also has applications that extend to other classes of objects, such as ultrametrics and partial orderings, presenting new methods for phylogenetics and time-course analysis.

We have applied the new protocols and algorithms to counting and cataloging representations from DNA and assembling cDNA templates for allelic variants and splice patterns. We are presently developing methods that will extend its application to whole-genome assembly and single-cell RNA and mitochondria sequencing.

PUBLICATIONS


Our lab develops novel computational analysis methods to study the structure and function of genomes, especially genomes with medical or agricultural significance. These include methods for assembling sequence data into complete genomes, aligning sequences to discover variations or measure transcription levels, and methods 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, in order 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 had several visiting members in addition to a vibrant group of students, postdocs, and analysts. Marley Alford was an undergraduate researcher in mathematics, joining us from Bard College. She worked closely with Maria Nattestad to develop novel methods for studying oncogene amplifications, especially to reconstruct the HER2 amplification within the SKBR3 breast cancer cell line. She received a full scholarship to present her work at the prestigious ABRCMS conference in the fall. Philipp Rescheneder joined us during the summer from his graduate program in Austria to work closely with Fritz Sedlazeck to develop novel methods for aligning high-error-rate single-molecule sequencing data, such as from PacBio or Oxford Nanopore sequencing. We are especially interested in using these developments to enhance the detection and analysis of structural variations in cancer. In addition, Hayan Lee successfully defended her Ph.D. thesis in computer science from Stony Brook University on “Algorithms and applications in genome assembly using long read sequencing technology.” After graduation, she moved to the Department of Energy Joint Genome Institute in Walnut Creek, California, as a postdoctoral fellow.

In the past year, we published several significant methods for de novo genome assembly, single-cell variant detection, and related high-throughput sequencing assays. These include an open-source hybrid error correction and de novo genome assembly algorithm for the new Oxford Nanopore MinION sequencer. We used the algorithm to produce the first de novo assembly of a eukaryotic genome using the handheld instrument. Another significant advance was publishing the first interactive analysis suite for single-cell copy number variations called Ginkgo. Using Ginkgo, researchers can easily identify copy number gains and losses presented within individual cells, as well as explore the population structure of complex tissues, such as the clonal populations within a tumor. Other advances include the publication of Metassembler, a pipeline to automatically merge together multiple assemblies of a genome into a single superior sequence, and our read mapping benchmarking and optimization suite Teaser. This provides a push-button solution for evaluating how different read mapping algorithms perform with different genome or read characteristics. We find this analysis is essential for accurately identifying variants with short reads, especially in non–model organisms that often have lower-quality assemblies, higher rates of heterozygosity, and other confounding factors. In addition to these algorithmic advances, we applied our technologies to publish the de novo genome assemblies of three major species: the pineapple (Ananas comosus), the Asian sea bass (Lates calcarifer), and an important regenerative flatworm (Macrostomum lignano). For this and other work, Schatz was awarded an Alfred P. Sloan Foundation Research Fellowship in Computational and Evolutionary Molecular Biology.

**Oxford Nanopore Sequencing, Hybrid Error Correction, and De Novo Assembly of a Eukaryotic Genome**

Monitoring the progress of DNA molecules through a membrane pore has been postulated as a method for sequencing DNA for several decades. Recently, a nanopore-based sequencing instrument, the Oxford Nanopore MinION, has become available, and we used this for sequencing the *Saccharomyces cerevisiae*
Research

To make use of these data, we developed a novel open-source hybrid error correction algorithm (Nanocorr) specifically for Oxford Nanopore reads, because existing packages were incapable of assembling the long read lengths (5–50 kbp) at such high error rates (between 5% and 40% error). With this new method, we were able to perform a hybrid error correction of the nanopore reads using complementary MiSeq data and produce a de novo assembly that is highly contiguous and accurate: The contig N50 length is more than 10 times greater than an Illumina-only assembly (678 kbp vs. 59.9 kbp) and has >99.88% consensus identity when compared with the reference. Furthermore, the assembly with the long nanopore reads presents a much more complete representation of the features of the genome, and correctly assembles gene cassettes, ribosomal RNAs (rRNAs), transposable elements, and other genomic features that were almost entirely absent in the Illumina-only assembly.

Interactive Analysis and Assessment of Single-Cell Copy Number Variations

We present Ginkgo (http://qb.cshl.edu/ginkgo), a user-friendly, open-source web platform for the analysis of single-cell copy number variations (CNVs). Ginkgo automatically constructs copy number profiles of cells from mapped reads and constructs phylogenetic trees of related cells. We validated Ginkgo by reproducing the results of five major studies. After comparing three commonly used single-cell amplification techniques, we concluded that degenerate oligonucleotide-primed polymerase chain reaction (PCR) is the most consistent for CNV analysis.

Teaser: Individualized Benchmarking and Optimization of Read Mapping Results for Next-Generation Sequencing Data

Mapping reads to a genome remains challenging, especially for non–model organisms with lower-quality assemblies or for organisms with higher mutation rates. Whereas most research has focused on speeding up the mapping process, little attention has been paid to optimizing the choice of mapper and parameters for a user’s data set. Here, we present Teaser, software that assists in these choices through rapid automated benchmarking of different mappers and parameter settings for individualized data. Within minutes, Teaser completes a quantitative evaluation of an ensemble of mapping algorithms and parameters. We use Teaser to show how Bowtie2 can be optimized for different data.

Metassembler: Merging and Optimizing De Novo Genome Assemblies

Genome assembly projects typically run multiple algorithms in an attempt to find the single best assembly, although those assemblies often have complementary, if untapped, strengths and weaknesses. We present our Metassembler algorithm that merges multiple assemblies of a genome into a single superior sequence. We apply it to the four genomes from the Assemblathon competitions and show it consistently and substantially improves the contiguity and quality of each assembly. We also develop guidelines for meta-assembly by systematically evaluating 120 permutations of merging the top five assemblies of the first Assemblathon competition.

The Pineapple Genome and the Evolution of CAM Photosynthesis

Pineapple (Ananas comosus (L.) Merr.) is the most economically valuable crop possessing crassulacean acid metabolism (CAM), a photosynthetic carbon assimilation pathway with high water-use efficiency, and the second most important tropical fruit. We sequenced the genomes of pineapple varieties F153 and MD2 and a wild pineapple relative, Ananas bracteatus accession CB5. The pineapple genome has one fewer ancient whole-genome duplication event than sequenced grass genomes, and a conserved karyotype with seven chromosomes from before the $\rho$ duplication event. The pineapple lineage has transitioned from C3 photosynthesis to CAM, with CAM-related genes showing a diel expression pattern in photosynthetic tissues. CAM pathway genes were enriched with cis-regulatory elements associated with the regulation of circadian clock genes, providing the first cis-regulatory link between CAM and circadian clock regulation. Pineapple CAM photosynthesis evolved by the reconfiguration of pathways in C3 plants, through the regulatory neofunctionalization of preexisting genes and not through the acquisition of neofunctionalized genes via whole-genome or tandem gene duplication.
Genome and Transcriptome of the Regeneration-Competent Flatworm, *M. lignano*

The free-living flatworm *M. lignano* has an impressive regenerative capacity. Following injury, it can regenerate almost an entirely new organism because of the presence of an abundant somatic stem cell population, the neoblasts. This set of unique properties makes many flatworms attractive organisms for studying the evolution of pathways involved in tissue self-renewal, cell-fate specification, and regeneration. The use of these organisms as models, however, is hampered by the lack of well-assembled and annotated genome sequences, fundamental to modern genetic and molecular studies. Here, we report the genomic sequence of *M. lignano* and an accompanying characterization of its transcriptome. The genome structure of *M. lignano* is remarkably complex, with ~75% of its sequence comprising simple repeats and transposon sequences. This has made high-quality assembly from Illumina reads alone impossible (N50 = 222 bp). We therefore generated 130× coverage by long sequencing reads from the Pacific Biosciences platform to create a substantially improved assembly with an N50 of 64 kbp. We complemented the reference genome with an assembled and annotated transcriptome and used both of these data sets in combination to probe gene-expression patterns during regeneration, examining pathways important to stem cell function.

Dual Functions of Macpiwi1 in Transposon Silencing and Stem Cell Maintenance in the Flatworm *M. lignano*

PIWI proteins and PIWI-interacting RNA (piRNA) pathways are essential for transposon silencing and some aspects of gene regulation during animal germline development. In contrast to most animal species, some flatworms also express PIWIs and piRNAs in somatic stem cells, where they are required for tissue renewal and regeneration. Here, we have identified and characterized piRNAs and PIWI proteins in the emerging model flatworm *M. lignano*. We found that *M. lignano* encodes at least three PIWI proteins. One of these, Macpiwi1, acts as a key component of the canonical piRNA pathway in the germline and in somatic stem cells. Knockdown of Macpiwi1 dramatically reduces piRNA levels, derepresses transposons, and severely impacts stem cell maintenance. Knockdown of the piRNA biogenesis factor Macvasa caused an even greater reduction in piRNA levels with a corresponding increase in transposons. Yet, in Macvasa knockdown animals, we detected no major impact on stem cell self-renewal. These results may suggest stem cell maintenance functions of PIWI proteins in flatworms that are distinguishable from their impact on transposons and that might function independently of what are considered canonical piRNA populations.

Molecular Genetic Diversity and Characterization of Conjugation Genes in the Fish Parasite *Ichthyophthirius multifilis*

*Ichthyophthirius multifilis* is the etiologic agent of “white spot,” a commercially important disease of freshwater fish. As a parasitic ciliate, *I. multifilis* infects numerous host species across a broad geographic range. Although *Ichthyophthirius* outbreaks are difficult to control, recent sequencing of the *I. multifilis* genome has revealed a number of potential metabolic pathways for therapeutic intervention, along with likely vaccine targets for disease prevention. Nonetheless, major gaps exist in our understanding of both the life cycle and population structure of *I. multifilis* in the wild. For example, conjugation has never been described in this species, and it is unclear whether *I. multifilis* undergoes sexual reproduction, despite the presence of a germline micronucleus. In addition, no good methods exist to distinguish strains, leaving phylogenetic relationships among geographic isolates completely unresolved. Here, we compared nucleotide sequences of SSUrDNA, mitochondrial NADH dehydrogenase subunit I and cox-1 genes, and 14 somatic single-nucleotide polymorphism (SNP) sites from nine *I. multifilis* isolates obtained from four different states in the United States since 1995. The mitochondrial sequences effectively distinguished the isolates from one another and divided them into at least two genetically distinct groups. Furthermore, none of the nine isolates shared the same composition of the 14 somatic SNP sites, suggesting that *I. multifilis* undergoes sexual reproduction at some point in its life cycle. Finally, compared with the well-studied free-living ciliates *Tetrahymena thermophila* and *Paramecium tetraurelia*, *I. multifilis* has lost 38% and 29%, respectively, of 16...
experimentally confirmed conjugation-related genes, indicating that mechanistic differences in sexual reproduction are likely to exist among *I. multifiliis* and other ciliate species.

**Biological Data Sciences in Genome Research**

The last 20 years have been a remarkable era for biology and medicine. One of the most significant achievements has been the sequencing of the first human genomes, which has laid the foundation for profound insights into human genetics, the intricacies of regulation and development, and the forces of evolution. Incredibly, as we look into the future over the next 20 years, we see the very real potential for sequencing more than one billion genomes, bringing even deeper insight into human genetics, as well as the genetics of millions of other species on the planet. Realizing this great potential for medicine and biology, though, will only be achieved through the integration and development of highly scalable computational and quantitative approaches that can keep pace with the rapid improvements to biotechnology. In this perspective, I aim to chart out these future technologies, anticipate the major themes of research, and call out the challenges ahead. One of the largest shifts will be in the training used to prepare the class of 2035 for their highly interdisciplinary world.

**Extending Reference Assembly Models**

The human genome reference assembly is crucial for aligning and analyzing sequence data and for genome annotation, among other roles. However, the models and analysis assumptions that underlie the current assembly need revising to fully represent human sequence diversity. Improved analysis tools and updated data reporting formats are also required.

**The Challenge of Small-Scale Repeats for Insertion and Deletion Discovery**

Repetitive sequences are abundant in the human genome. Different classes of repetitive DNA sequences, including simple repeats, tandem repeats, segmental duplications, interspersed repeats, and other elements, collectively span >50% of the genome. Because repeat sequences occur in the genome at different scales, they can cause various types of sequence analysis errors, including in alignment, de novo assembly, and annotation, among others. This mini-review highlights the challenges introduced by small-scale repeat sequences, especially near-identical tandem or closely located repeats and short tandem repeats, for discovering DNA insertion and deletion (indel) mutations from next-generation sequencing data. We also discuss the de Bruijn graph sequence assembly paradigm that is emerging as the most popular and promising approach for detecting indels. The human exome is taken as an example and highlights how these repetitive elements can obscure or introduce errors while detecting these types of mutations.

**Big Data: Astronomical or Genomical?**

Genomics is a big data science and is going to get much bigger, very soon, but it is not known whether the needs of genomics will exceed other big data domains. Projecting to the year 2025, we compared genomics with three other major generators of big data: astronomy, YouTube, and Twitter. Our estimates show that genomics is a “four-headed beast”—it is either on par with or the most demanding of the domains analyzed here in terms of data acquisition, storage, distribution, and analysis. We discuss aspects of new technologies that will need to be developed to rise up and meet the computational challenges that genomics poses for the near future. Now is the time for concerted, community-wide planning for the “genomical” challenges of the next decade.

**PUBLICATIONS**


POPULATION GENETICS AND TRANSCRIPTIONAL REGULATION

A. Siepel  N. Dukler  M. Hubisz  L. Pipes
B. Gulko  E. Hutton  A. Platts
Y. Huang  J. Mohammed  R. Ramani

For the past several years, our research has focused in two major areas: human population genetics and transcriptional regulation in humans and Drosophila. The research in population genetics is performed either with publicly available genomic sequence data or with a variety of collaborators, whereas most of the work on transcriptional regulation is performed with our collaborator John Lis at Cornell. We also have smaller collaborative projects on topics ranging from comparative transcriptomics of primates (with Chris Mason, Weill Cornell Medical College), to molecular evolution of micro-RNAs in Drosophila (with Eric Lai, Memorial Sloan Kettering Cancer Center), to prediction of the fitness consequences (fitCons) of mutations in rice and other crops (with Michael Purugganan, NYU), to studying the speciation process of recently diverged Sporophila songbirds (with John “Irby” Lovette, Cornell, and Ilan Gronau, Herzliya Interdisciplinary Center, Israel), to combined experimental and computational characterization of cis-regulatory sequences in the human genome (with Barak Cohen, Washington University, St. Louis). We focus on theoretical and computational research and do not generate our own data, but we often work closely with experimental collaborators on projects that have substantial experimental, as well as computational, components. We are broadly interested in molecular evolution, population genetics, and gene regulation, as well as machine learning, probabilistic modeling, and Bayesian statistics, and our research projects cut a broad swath across these diverse areas. The group is highly interdisciplinary, with members trained in computer science, mathematics, physics, genetics, and biochemistry, among other areas.

Since our move to CSHL from Cornell in September 2014, we have expanded considerably. New members of the group this year include postdoctoral researcher Yifei Huang, research associate Adrian Platts, programmer Ritika Ramani, and Watson School Ph.D. student Elizabeth (Lizzie) Hutton. Ph.D. student Brad Gulko also recently moved from Cornell to CSHL to finish his dissertation here. Our group has continued to push forward with three core research projects that we have been working on for several years, as discussed below. In addition, we have launched several exciting new projects that are still in the early stages of development, but which we hope to discuss in future reports.

Reconstruction of Demographic History from Complete Genome Sequences

Several years ago, we developed a statistical method based on the theoretical framework of the coalescent for reconstructing the demographic history of complex, structured populations from DNA sequence data. Our method, called G-PhoCS (Generalized Phylogenetic Coalescent Sampler), uses Markov chain Monte Carlo techniques to explore coalescent genealogies consistent with a particular population phylogeny, allowing for gene flow among designated populations. G-PhoCS produces Bayesian estimates of the key parameters that define these population phylogenies, such as the divergence times among populations, the effective sizes of ancestral populations, and the rates of postdivergence gene flow. We originally used the method to estimate the date of origin of one of the earliest branching extant human populations, the Khoisan hunter–gatherers of southern Africa. More recently, we have used it in collaboration with other research groups to shed light on the demographic histories of dogs and wild canids, and birds from the genus Sporophila (e.g., Campagna et al. 2015).

G-PhoCS and methods like it “cheat” by considering only short, widely spaced genomic sequences and ignoring the difficult problem of modeling recombination. However, it would be preferable to consider not only the process of coalescence (finding common ancestry) at each locus in a genome, but also the manner in which historical recombination events alter these genealogies along the genome sequence. This
combined history of coalescence and recombination can be explicitly represented by a generalized representation known as an "ancestral recombination graph," or ARG. The problem of reconstructing an ARG from sequence data, however, is notoriously difficult, and ARG inference has not been widely used in applied population genomics. Recently, we developed an algorithm for sampling ARGs within an approximate framework known as the sequentially Markov coalescent (SMC). Our method, called ARGweaver, uses techniques from hidden Markov models to repeatedly “thread” individual sequences through an ARG, leading to a Gibbs sampler over the space of ARGs. ARGweaver is the first ARG inference method that is efficient enough to apply to complete mammalian genomes. We have shown that it works remarkably well on simulated data and that it reveals clear signatures of natural selection in real human genome sequences.

This year, together with collaborators from the Max Planck Institute for Evolutionary Anthropology, in Leipzig, Germany, we published a major paper on human evolutionary history that made heavy use of both G-PhoCS and ARGweaver (Kuhlwilm et al. 2016). Using these new computational tools, we were able to perform the first joint analysis of multiple archaic human genomes and multiple modern genomes and propose a single unified demographic model that explains all of the patterns of genetic variation observed in these sequences. This model includes previously reported gene flow events between archaic and modern humans, including the now-famous event, dated to 47–65 thousand years ago, that is responsible for the 1%–3% of Neanderthal DNA found in human populations outside of Africa. Interestingly, however, this model also includes a much older gene flow event in the opposite direction, explaining the presence of early modern human genome sequences in one of the Neanderthal genomes. This event dates back to more than 100,000 years ago and appears to require an earlier out-of-Africa migration of modern humans than the migration event 50,000–60,000 years ago that is mainly responsible for the peopling of Europe and Asia. Therefore, our findings support not only an additional interbreeding event between archaic and modern humans, resulting in gene flow in the opposite direction of previously reported events, but they also support a scenario with multiple migrations by early modern humans out of Africa. To show that this scenario is the most plausible explanation for the observed patterns in the data, we performed three separate genome-wide analyses—one using G-PhoCS, one using ARGweaver, and a third based on patterns of heterozygosity and genetic divergence in large windows along the genome. Our paper describing this analysis generated a great deal of interest in the scientific community and was widely reported in the press.

**Analysis of Natural Selection on Regulatory Sequences in the Human Genome**

We have a long-standing interest in characterizing the influence of natural selection on DNA sequences, particularly in noncoding regions of the genome. Most of our work in this area has involved comparisons of complete mammalian genomes and, hence, has considered evolutionary processes spanning tens to hundreds of millions of years. More recently, however, we have become interested in integrating this phylogenetic information with data on human polymorphism, to gain insight into more recent evolutionary events. A few years ago, we developed a probabilistic model and inference method, called INSIGHT, that makes use of joint patterns of divergence and polymorphism to shed light on recent natural selection. INSIGHT focuses, in particular, on disentangling the influence of positive and negative selection on collections of short, interspersed genomic elements. We have used INSIGHT to show that natural selection has profoundly influenced transcription factor binding sites across the genome during the past 5,000,000 years of evolution, with major contributions both to adaptive changes in humans and to weakly deleterious variants currently segregating in human populations.

The INSIGHT method provides an estimate of the fraction of nucleotides under natural selection in any given collection of genomic elements. This same estimate can alternatively be interpreted as probabilities that mutations falling in the given elements will have fitCons for the organisms that carry them. We recently realized that this property could be used to produce fitCons scores across the entire human genome. Using high-throughput data from the ENCODE project, we first partition the genome into classes of sites having characteristic functional genomic “fingerprints” in a given cell type. We then use INSIGHT to calculate a fitCons score for each fingerprint (Gulko et al. 2015).
Finally, we plot these scores along the genome sequence. These fitCons scores turn out to be remarkably powerful for identifying unannotated regulatory elements in the human genome. We have also used fitCons scores to estimate that only ~7% of nucleotides in the human genome directly influence fitness. Work is under way to extend these methods to accommodate much larger functional genomic data sets and to apply them to other cell types and organisms. Plant biologists are particularly interested in the potential of these methods to guide breeding designs for crops to improve yield, drought tolerance, and other desirable traits.

Characterization of Transcriptional Regulation Using Global Run-On and Sequencing

For several years, we have been working closely with John Lis’s group on methods for interpreting data generated using their powerful GRO-seq (global run-on and sequencing) technology, which maps the positions of engaged RNA polymerases across the genome. It has gradually become clear that an unanticipated benefit of GRO-seq and derived technologies is that they are uniquely well suited for detecting so-called enhancer RNAs (or eRNAs) and, consequently, for identifying active enhancers and other regulatory elements in mammalian cells. Last year, we systematically compared patterns in DNA sequences and chromatin near the sites of transcription initiation in both annotated genes and regulatory elements. We found that the architecture of transcription initiation was remarkably similar at these regions and proposed a unified model for enhancers and promoters. Under this model, the key distinctions between these regions occur in downstream steps, which cause protein-coding messenger RNAs (mRNAs) to become stable, whereas other RNAs are rapidly degraded by the cell. This year, we published a new machine-learning method, called dREG, for detecting regulatory elements from standard GRO- or PRO-seq data, based on their characteristic patterns of transcription initiation. As a result, it is now possible to detect regulatory elements and measure transcriptional activity using a single experimental assay.

In close collaboration with former postdoc Charles Danko, who led development of dREG and is now on the faculty at Cornell, we are now using dREG to analyze PRO-seq data from humans, chimpanzees, and rhesus macaques to shed light on the evolution of gene regulation in primates. In another project, we are using similar methods to examine the dynamics of regulatory cascades in K562 cells based on time courses of PRO-seq data after induction with a small molecule called celastrol.

PUBLICATIONS


In Press

In 1986, Cold Spring Harbor Laboratory began a Fellows Program to encourage independent research by outstanding young scientists who, during their graduate studies, displayed exceptional promise of becoming leading scientists of the future. The purpose of this program is to provide an opportunity for these young scientists to work independently at the Laboratory for a period of 3 years on projects of their own choosing. Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free of distraction. The interactions among research groups at the Laboratory and the program of courses and meetings on diverse topics in biology contribute to a research environment that is ideal for innovative science by these Fellows. The CSHL Fellows Program has been tremendously successful and has served as a paradigm for several analogous programs at other institutions, most recently a Fellows Program sponsored by the National Institutes of Health.

The success of the program is apparent from the list of distinguished alumni. Most notably, Carol Greider—recipient of the 2009 Nobel Prize in Physiology or Medicine for her work on telomerase and telomere function—joined the Fellows Program in 1998. After completing her fellowship, Carol was on the CSHL faculty for 9 years, and she is currently the Daniel Nathans Professor and Director of Molecular Biology and Genetics at Johns Hopkins University School of Medicine.

Previous CSHL Fellows Adrian Krainer (1986) and Marja Timmermans (1998) are now Professors at the Laboratory, and Chris Vakoc (2008) and Florin Albeanu (2008) are currently holding Assistant Professor positions at CSHL. Scott Lowe (1995) is a Howard Hughes Medical Institute (HHMI) Investigator. After nearly 15 years on the CSHL faculty, he recently took on a Professorship at Memorial Sloan-Kettering Cancer Center in New York City. Eric Richards (1989) currently is the Vice President of Research and Senior Scientist at the Boyce Thompson Institute for Plant Research at Cornell University; David Barford (1991) is a Fellow of the Royal Society and Professor of Molecular Biology at the Institute of Cancer Research in London; Ueli Grossniklaus (1994) is Professor at the Institute of Plant Biology, University of Zürich, Switzerland; and Térence Strick (2000) left at the end of his fellowship to become a Group Leader at the Institute Jacques Monod in Paris. Lee Henry (2000) joined HHMI’s Janelia Farm in Ashburn and joined a project headed by Thomas Südhof. Ira Hall (2004) is an Associate Professor and Associate Director of the Genome Institute at Washington University. Patrick Paddison, who had joined the Fellows Program in 2004, currently is an Associate Member at the Fred Hutchinson Cancer Research Center in Seattle, Washington.

Lingbo Zhang has been a Fellow at the Laboratory since 2013. He joined us from Harvey Lodish’s laboratory at the Whitehead Institute of the Massachusetts Institute of Technology, where he studied the regulation of erythroid progenitor cell self-renewal. As a CSHL Fellow, Lingbo is conducting genetic and small-molecule screens to discover novel regulators of normal and aberrant stem cell biology.
Self-renewal is a unique property of stem and progenitor cells, allowing them to undergo multiple cycles of cell division while maintaining an undifferentiated status. During normal development, self-renewal allows adult stem and progenitor cells to maintain tissue homeostasis. In cancer, malignant cells hijack the self-renewal property to form cancer stem cells (or tumor-initiating cells) to contribute to carcinogenesis.

Our laboratory is interested in understanding the molecular mechanisms underlying self-renewal of both normal and malignant stem and progenitor cells. We are probing the mechanistic divergence of self-renewal in normal versus malignant stem and progenitor cells to develop a targeted therapy that selectively disrupts carcinogenesis but not normal tissue homeostasis. As abnormal metabolism is emerging as a hallmark of cancer, we have a specific focus on how metabolic and energetic changes are involved in the regulation of self-renewal with the ultimate goal to uncover metabolic vulnerabilities for cancer treatment.

The research in our laboratory focuses on the hematopoietic, or blood-forming, system, using this lineage as a model system to uncover mysteries behind adult and malignant stem and progenitor self-renewal. Currently, we are mainly studying two important cell types, the early erythroid progenitor and the leukemia stem cell. Using both CRISPR-Cas9 functional genomics and chemical biology approaches, we are developing small chemical compound–based therapeutic strategies for blood malignancies, including myelodysplastic syndrome (MDS) and leukemia.

**Targeting Early Erythroid Progenitor Self-Renewal as Novel Therapeutic Strategies for MDS**

MDS is a form of lethal hematopoietic malignancy characterized by pancytopenia resulting from progressive bone marrow failure. Anemia is a major manifestation of MDS that results in patient death. The treatment option for MDS is limited. Although a small portion of MDS patients respond to erythropoietin (EPO) and lenalidomide, the only therapeutic option for the majority of MDS patients is frequent blood transfusion, which causes iron overload toxicity and organ failure. We therefore focus our research on the highly self-renewing early erythroid progenitor population, whose failure causes EPO unresponsiveness, to harness its expansion and regeneration potential for the formation of thousands of erythrocytes in the treatment of resistant MDS.

We combined in silico gene expression analysis and a forward chemical genomic approach and identified several novel regulators of early erythroid progenitor self-renewal. Blocking these targets by either short hairpin RNAs (shRNAs) or chemical probes triggered expansion of primary early erythroid progenitors ex vivo. We further focused on one novel regulator and tested its efficacies in a preclinical conditional knock-in Cre-Mx1 Srsf2 P95H/WT MDS mouse model. We showed that injecting a drug-like small chemical probe completely corrected anemia of MDS and extended the survival of MDS mice to a level the same as control wild-type mice, both of which suggested that this is not just a treatment but a cure for anemia of MDS. We are currently developing this promising drug candidate with the ultimate goal of entering clinical trials for refractory MDS.

**Targeting Metabolic Divergences between Leukemic Cells and Normal Hematopoietic Stem and Progenitor Cells as Novel Therapeutic Strategies for Leukemia**

Abnormal metabolic pathways are an emerging hallmark of cancer. Cancer cells undergo extensive reprogramming to fulfill metabolic and energetic requirements to support their abnormal proliferation.
The lack of understanding of molecular details precludes the invention of novel therapies for cancer through targeting these metabolic changes. More importantly, normal stem and progenitor cells in adult tissues, such as hematopoietic stem and progenitor cells (HSPCs), also undergo regular expansion and proliferation; therefore, HSPCs share similar metabolic requirements as cancer cells, such as the extensive formation of macromolecules to generate building blocks for daughter cells.

Our group is beginning to identify metabolic divergences between normal HSPCs and malignant leukemic cells. By using a CRISPR-Cas9 functional genomic approach, we screened approximately 300 genes encoding metabolic enzymes and transporters. Approximately 20 genes have been identified as positive hits. We are currently validating these genes and corresponding chemical probes in both primary leukemic cell culture systems and genetically engineered leukemia mouse models as novel drug targets to treat leukemia.
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Linda Van Aelst

Members
W. Richard McCombie
Stephen Shea
Christopher Vakoc

EXTERNAL ADVISORY COMMITTEE

Keith Yamamoto (Chair)
Executive Vice Dean, School of Medicine
University of California, San Francisco

Victor Corces
Professor, Department of Biology
Emory University
Professor, Howard Hughes Medical Institute

Gail Mandel
Senior Scientist, Vollum Institute
Oregon Health and Science University
Investigator, Howard Hughes Medical Institute

Marguerite Mangin
Academic Programs Director and Senior Research Associate
The Rockefeller University

Barbara Meyer
Professor of Genetics and Development
University of California, Berkeley
Investigator, Howard Hughes Medical Institute

Frank Solomon
Professor, Department of Biology and Center for Cancer Research
Massachusetts Institute of Technology
The 12th WSBS Graduation

On April 19, 2015, we celebrated the Watson School’s 12th graduation ceremony. Twelve students were awarded Ph.D. degrees: Ozlem (Mert) Aksoy, Mitchell Bekritsky, Sang-Geol Koh, Nilgun Tasdemir, Elvin Wagenblast, and Susann Weissmueller from the Entering Class of 2008, Stephane Castel, Kristen Delevich, Wee Siong Goh, Ian Peikon, and Cinthya Zepeda Mendoza from the Entering Class of 2009, and Jack Walleshauser from the Entering Class of 2010. Michael Giangrasso from the Entering Class of 2013 was awarded a Masters degree, but did not attend the ceremony. Honorary degrees were bestowed upon three deserving individuals: Mr. Charlie Rose, Lord David Sainsbury, and Dr. Hannah Gray.

Charlie Rose is the anchor and executive editor of Charlie Rose, a nationally syndicated television program, which has aired on PBS since the start of 1993. He has interviewed extraordinary men and women of science, politics, art, business, sports, technology, literature, and entertainment. He has done a number of series on science, particularly his series on the brain, that offer the general public a much richer and more sophisticated insight into the scientific endeavor, as well as a more nuanced take on the future of medical advances. Rose also co-anchors CBS This Morning and is a contributing correspondent to 60 Minutes.

David Sainsbury was a former Chairman of J. Sainsbury plc, the supermarket chain established by his great-grandfather in 1869. Later, he served as a Minister of Science in the last British Labour government. In his role as philanthropist, Sainsbury established the Gatsby
Watson School of Biological Sciences

Charitable Foundation. With a particular emphasis on plant science and neuroscience, the Gatsby Foundation has had a major impact on biological research in the United Kingdom. In recognition of his achievements and service, David Sainsbury was elected an honorary Fellow of the Royal Society, was elected Chancellor of the University of Cambridge, and was made Lord Sainsbury of Turville in 1997.

Hannah Gray served as President of the University of Chicago for 15 years until 1993. She remains at Chicago as President Emeritus and the Harry Pratt Judson

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### 2015 WSBS DOCTORAL RECIPIENTS

<table>
<thead>
<tr>
<th>Student</th>
<th>Thesis advisor</th>
<th>Academic mentor</th>
<th>Current position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colleen Carlston</td>
<td>Christopher Hammell</td>
<td>Hiro Furukawa</td>
<td>Clinical Molecular Genetics Fellow, University of Utah</td>
</tr>
<tr>
<td>Silvia Fenoglio</td>
<td>Gregory Hannon</td>
<td>Linda Van Aelst</td>
<td>Postdoctoral Fellow, Massachusetts Institute of Technology (Advisor: Michael Hemann)</td>
</tr>
<tr>
<td>Wee Siong Goh</td>
<td>Gregory Hannon</td>
<td>Hiro Furukawa</td>
<td>Research Fellow, Institute of Molecular and Cellular Biology, Singapore (Advisor: William Burkholder)</td>
</tr>
<tr>
<td>Jack Walleshauser</td>
<td>Leemor Joshua-Tor</td>
<td>Christopher Hammell</td>
<td>Postdoctoral Fellow, Yale University (Advisor: Anna Pyle)</td>
</tr>
</tbody>
</table>

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### 2015 THESIS DISSERTATION DEFENSES

#### ENTERING CLASS OF 2009

**Silvia Fenoglio,** May 27, 2015

*Context-specific dependencies in a mouse model of pancreatic cancer.*

**Thesis Examining Committee**

- Chair: Raffaella Sordella
- Research Mentor: Gregory Hannon
- Academic Mentor: Linda Van Aelst
- Committee Member: Michael Hemann, Massachusetts Institute of Technology
- Committee Member: Scott Lowe
- External Examiner: Andrea Ventura, Memorial Sloan Kettering Cancer Center

**Identification and characterization of lin-4 suppressors via a genome-wide C. elegans RNAi screen including the novel heterochronic gene pqn-59.**

**Thesis Examining Committee**

- Chair: Josh Dubnau
- Research Mentor: Christopher Hammell
- Academic Mentor: Hiro Furukawa
- Committee Member: Marja Timmermans
- External Examiner: Allison Abbott, Marquette University

**Jack Walleshauser,** March 18, 2015

*A novel technology for the space-specific recovery of biological molecules.*

**Thesis Examining Committee**

- Chair: Gregory Hannon
- Research Mentor: Leemor Joshua-Tor
- Academic Mentor: Christopher Hammell
- Committee Member: Adrian Kraner
- External Examiner: Anna Marie Pyle, Yale University

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**Colleen Carlston,** June 17, 2015

**ENTRERING CLASS OF 2010**
Distinguished Service Professor of History Emeritus. Dr. Gray has served on the governing boards of many nonprofit institutions including the Harvard University Corporation, the Yale University Corporation, the Smithsonian Institution, and Bryn Mawr College. She was Chair of the Andrew W. Mellon Foundation and of the Howard Hughes Medical Institute. Under Dr. Gray’s leadership, the Howard Hughes Medical Institute expanded, including building its first stand-alone research institute, the very successful Janelia Farm Research Campus, as well as enhancing the Science Education Program. Dr. Gray is the recipient of a Medal of Liberty, the Presidential Medal of Freedom, and the Jefferson Medal of the American Philosophical Society and the National Humanities. She holds honorary degrees from Harvard, Oxford, Princeton, and Columbia Universities, among many others.

Dr. Gray delivered an inspiring and entertaining commencement address. In acknowledging the Lab’s 125th anniversary, she observed that “this year’s graduates comprise an anniversary class that demonstrates what Cold Spring is all about in the range of its science and in its role of helping to shape the future as well as the history of the biomedical sciences.”

As with each graduation, we extended a special welcome to the family members and friends of our students who attended the ceremony.

Teaching Award

At the graduation ceremony this year, WSBS Associate Professor Bo Li was presented with the 10th annual Winship Herr Faculty Teaching Award, named in honor of the School’s founding Dean. Bo is the instructor of the Scientific Reasoning and Logic module on Neuroscience and was chosen by the students for this award, based on his enthusiasm and creativity in teaching. Here is some of what the students said about Bo’s teaching in their nominations:

“Bo was really good at conveying the essentials of a subject I knew very little about prior to the course. His lecturing style was concise and easy to follow.”

“Bo was incredibly organized during the course, clearly delineating the expectations and scope of the course early on. He was also encouraging and helpful when a concept wasn’t well understood.”

Faculty Changes

One new faculty member joined the Watson School in 2015: Camila dos Santos. A former postdoctoral fellow from Gregory Hannon’s laboratory, Assistant Professor Camila dos Santos studies how the signals present during pregnancy permanently alter the way gene expression is controlled and how these changes affect normal and malignant mammary development. She has already participated in WSBS activities, including giving a Research Topics talk to the first-year students and judging the poster session at the In-House Symposium. We look forward to her growing participation as a member of the faculty.

Admissions 2015

The School received 310 applications for Entering Class of 2015 and is indebted to its Admissions Committee, which reviewed, interviewed, and selected candidates for our doctoral program.
## DOCTORAL THESIS RESEARCH

### ENTERING CLASS OF 2010

<table>
<thead>
<tr>
<th>Student</th>
<th>Academic mentor</th>
<th>Research mentor</th>
<th>Thesis research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arkarup Bandyopadhyay</td>
<td>Zachary Lippman</td>
<td>Florin Albeanu</td>
<td>Identity and intensity encoding of odors in rodents.</td>
</tr>
<tr>
<td>Goldberg Lindsay Fellow</td>
<td>Bo Li</td>
<td>Florin Albeanu</td>
<td>Roles of olfactory bulb inhibitory microcircuits in shaping the temporal response properties of mitral cells.</td>
</tr>
<tr>
<td>Matthew Koh</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>George A. and Marjorie H. Anderson Fellow</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NIH Predoctorial Trainee</td>
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</table>

### ENTERING CLASS OF 2011

<table>
<thead>
<tr>
<th>Student</th>
<th>Academic mentor</th>
<th>Research mentor</th>
<th>Thesis research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert Aboukhalil</td>
<td>Josh Dubnau</td>
<td>Mickey Atwal, Michael Wigler</td>
<td>Using single-cell RNA-Seq to investigate tumor heterogeneity and evolution.</td>
</tr>
<tr>
<td>NIH Predoctoral Trainee</td>
<td></td>
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</tr>
<tr>
<td>Starr Centennial Scholar</td>
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<td></td>
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</tr>
<tr>
<td>Brittany Cazakoff</td>
<td>Christopher</td>
<td>Stephen Shea</td>
<td>Dynamic granule cell processing of odor information.</td>
</tr>
<tr>
<td>Edward and Martha Gerry Fellow</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NSERC Scholar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joaquina Delas Vives</td>
<td>Nicholas Tonks</td>
<td>Gregory Hannon</td>
<td>Functional role of long noncoding RNAs in hematopoiesis.</td>
</tr>
<tr>
<td>La Caixa Fellow</td>
<td></td>
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<tr>
<td>Boehringer Ingelheim Fonds Fellow</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Anja Hohmann</td>
<td>John Inglis</td>
<td>Christopher Vakoc</td>
<td>Exploring the role of bromodomain-containing protein 9 (Brd9) in the maintenance of acute myeloid leukemia.</td>
</tr>
<tr>
<td>David H. Koch Fellow</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Boehringer Ingelheim Fonds Fellow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Justus Kebschull</td>
<td>Marja Timmermans</td>
<td>Anthony Zador</td>
<td>Grasping the brain.</td>
</tr>
<tr>
<td>David and Fanny Luke Fellow</td>
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<tr>
<td>Genentech Foundation Fellow</td>
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<tr>
<td>Boehringer Ingelheim Fonds Fellow</td>
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<tr>
<td>Fred Marbach</td>
<td>Josh Dubnau</td>
<td>Anthony Zador</td>
<td>A study of auditory corticostriatal cells in the behaving mouse.</td>
</tr>
<tr>
<td>Farish-Gerry Fellow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onyekachi Odoemene</td>
<td>Stephen Shea</td>
<td>Anne Churchland</td>
<td>The role of neural inhibition in perceptual decision-making.</td>
</tr>
<tr>
<td>NIH Individual Fellowship</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>William Randolph Hearst Scholar</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sophie Thomain</td>
<td>Josh Dubnau</td>
<td>Zachary Lippman</td>
<td>Characterization of a new meristem maintenance pathway in tomato and <em>Arabidopsis thaliana</em> and its relation to pollen tube growth.</td>
</tr>
<tr>
<td>George A. and Marjorie H. Anderson Fellow</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Charles Underwood</td>
<td>Michael Schatz</td>
<td>Robert Martienssen</td>
<td>Epigenetic inheritance through mitosis and meiosis in <em>Arabidopsis thaliana</em>.</td>
</tr>
<tr>
<td>William R. Miller Fellow</td>
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</tbody>
</table>

### ENTERING CLASS OF 2012

<table>
<thead>
<tr>
<th>Student</th>
<th>Academic mentor</th>
<th>Research mentor</th>
<th>Thesis research</th>
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</thead>
<tbody>
<tr>
<td>Talitha Forcier</td>
<td>Nicholas Tonks</td>
<td>Justin Kinney</td>
<td>Building the transcriptional regulatory code from the ground up.</td>
</tr>
<tr>
<td>NIH Predoctoral Trainee</td>
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</tr>
<tr>
<td>William Randolph Hearst Scholar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tyler Garvin</td>
<td>Zachary Lippman</td>
<td>Michael Schatz</td>
<td>Structural variants and gene networks underlying complex human disease.</td>
</tr>
<tr>
<td>NIH Predoctoral Trainee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yu-Jui (Ray) Ho</td>
<td>Michael Schatz</td>
<td>Molly Hammell</td>
<td>Methods development for low-input RNA-Seq analysis and application to breast cancer heterogeneity.</td>
</tr>
<tr>
<td>David and Fanny Luke Fellow</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Paul Masset</td>
<td>Jan A. Witzkowski</td>
<td>Adam Kepecs</td>
<td>Representations of decision confidence in the brain: From Bayes’ rule to channelrhodopsin.</td>
</tr>
<tr>
<td>Florence Gould Fellow</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Annabel Romero Hernandez</td>
<td>Adrian R. Krainer</td>
<td>Hiro Furuwaka</td>
<td>Molecular mechanisms of inhibition in NMDA receptors.</td>
</tr>
<tr>
<td>Genentech Foundation Fellow</td>
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<tr>
<td>(continued)</td>
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</tr>
<tr>
<td>Student</td>
<td>Academic mentor</td>
<td>Research mentor</td>
<td>Thesis research</td>
</tr>
<tr>
<td>---------------------------------</td>
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</tr>
<tr>
<td>Abram Santana</td>
<td>Lloyd Trotman</td>
<td>David Tuveson</td>
<td>Pancreatic tumor biology and therapy.</td>
</tr>
<tr>
<td>ENTRING CLASS OF 2013</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Giorgia Battistoni</td>
<td>Christopher</td>
<td>Gregory Hannon</td>
<td>One-carbon metabolism and DNA methylome dynamics in pancreatic cancer.</td>
</tr>
<tr>
<td>Lital Charatisky</td>
<td>John Inglis</td>
<td>Anne Churchland</td>
<td>Neural circuits for multisensory integration in normal and disease states.</td>
</tr>
<tr>
<td>Sanchari Ghosh</td>
<td>Josh Dubnau</td>
<td>Anthony Zador</td>
<td>Role of corticostriatal plasticity in learning an auditory discrimination task.</td>
</tr>
<tr>
<td>Michael Gutbrod</td>
<td>Zachary Lippman</td>
<td>Robert Martienssen</td>
<td>Small RNA and the RNAi pathway in transposable element regulation and differentiation in the preimplantation embryo.</td>
</tr>
<tr>
<td>Daniel Kepple</td>
<td>David Stewart</td>
<td>Alexei Koulaikov</td>
<td>The human olfactory space.</td>
</tr>
<tr>
<td>Laura Maiorino</td>
<td>Nicholas Tonks</td>
<td>Mikala Egeblad</td>
<td>Understanding the role of the epithelial–mesenchymal plasticity in pancreatic cancer metastasis.</td>
</tr>
<tr>
<td>Maria Nattestad</td>
<td>Linda Van Aelst</td>
<td>Michael Schatz</td>
<td>Algorithms for analyzing and assembling complex genomes.</td>
</tr>
<tr>
<td>Georgi Yordanov</td>
<td>Leemor Joshua-Tor</td>
<td>David Tuveson</td>
<td>Role of c-Myc in pancreatic cancer.</td>
</tr>
<tr>
<td>ENTRING CLASS OF 2014</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Emillis Bruzas</td>
<td>Alea Mills</td>
<td>Mikala Egeblad</td>
<td>Investigation of mechanisms responsible for reawakening and chemoresistance in a breast cancer dormancy model.</td>
</tr>
<tr>
<td>Hamza Giaffar</td>
<td>Jan Wirkowski</td>
<td>Alexei Koulaikov</td>
<td>The primacy model of olfactory coding.</td>
</tr>
<tr>
<td>Jacqueline Giovanniello</td>
<td>Bruce Stillman</td>
<td>Bo Li</td>
<td>Disruption of central amygdala fear circuit in a 16p11.2 microdeletion model of autism.</td>
</tr>
<tr>
<td>Elizabeth Hutton</td>
<td>Molly Hammell</td>
<td>Adam Siepel</td>
<td>Functional variant prediction in noncoding regions.</td>
</tr>
<tr>
<td>Sashank Pisupati</td>
<td>Stephen Shea</td>
<td>Anne Churchland</td>
<td>Dissecting the circuits and mechanisms that support optimal multisensory integration in rodents.</td>
</tr>
<tr>
<td>Colin Stoneking</td>
<td>Zachary Lippman</td>
<td>Anthony Zador</td>
<td>Neuronal mechanisms enabling decision-making to be learned.</td>
</tr>
<tr>
<td>Jue Xiang Wang</td>
<td>Mikala Egeblad</td>
<td>Hiro Furuwaka</td>
<td>Impact of subunit composition and de novo mutations on NMDA receptor structure, channel function, and interactions.</td>
</tr>
<tr>
<td>Anqi Zhang</td>
<td>Bo Li</td>
<td>Anthony Zador</td>
<td>From corticostrial plasticity to a common pathway.</td>
</tr>
</tbody>
</table>
The Admissions Committee for the 2015 entering class comprised Adrian Krainer (chair), Mickey Atwal, Bo Li, Zachary Lippman, W. Richard McCombie, Stephen Shea, Nicholas Tonks, Christopher Vakoc, Linda Van Aelst, and myself.

**Entering Class of 2015**

On August 17, 2015, the WSBS welcomed the 17th incoming class. The seven students—Benjamin Berube, Kristina Grigaityte, Matt Lee, Katarina Meze, Alexandra Nowlan, Sofya Polyanskaya, and Ngoc Tran—hail from the United States, Lithuania, Russia, the United Kingdom, and Vietnam.

### ENTERING CLASS OF 2015

**Benjamin Berube,** Saint Anselm College: B.A. in Biochemistry (2015); NH-INBRE Undergraduate Research Fellow, Saint Anselm College (2013–2015); NH-INBRE Summer Undergraduate Research Fellow, Dartmouth College (2014); NH-INBRE Conference Student Speaker (2014); NH Science Olympiad Guest Speaker (2014); Biological National Honor Society, Beta Beta Beta (2013); Saint Anselm SOAR Event Student Speaker

*Academic Mentor: Zachary Lippman*

**Kristina Grigaityte,** University of Edinburgh: B.S. in Biomedical Sciences, Neuroscience Honours (2015); Summer Research Associate, University of California, Los Angeles (2014); Research Assistant, University of California, Los Angeles (2013)

*Academic Mentor: John Inglis*

**Matthew Lee,** University of Bristol: B.S. in Biochemistry (2015); Faculty Undergraduate Scholarship Award (2014); Harry Perkins Institute of Medical Research in Perth, Australia Summer Research (2014); Gold Duke of Edinburgh awarded by HRH Prince Philip at St. James Palace (2013)

*Academic Mentor: Nicholas Tonks*

**Katarina Meze,** University of York: B.S. in Chemistry (2015); Institute of Molecular Biology, University of Oregon Summer Research (2014); Engineering and Physical Sciences Research Council Summer Research Bursary (2013); Sandoz Summer Industrial Research Placement (2012); Chemistry Departmental Scholarship (2012)

*Academic Mentor: Jay Lee*

**Alexandra Nowlan,** University of Massachusetts, Amherst; B.S. in Biology (2011); Harvard University Extension School, Graduate Level Coursework, Neurobiology (2012); Magna Cum Laude (2011); Dean’s List Honors (2007–2011); Biology Talent Advancement Program (2007–2008); Junior Fellow in the Life Sciences (2010–2011); Commonwealth College Research Assistant Fellowship (2009–2011); HHMI Summer Fellowship in Biomedical Research, Williams College (2006); Williamstown Community Scholarship (2009–2010); John and Abigail Adams Scholarship (2007–2011)

*Academic Mentor: Jessica Tollkuhn*

**Sofya Polyanskaya,** Lomonosov Moscow State University (MSU): B.S. in Molecular Biology (2015); Winner of the Increased State Academic Scholarship, three separate awards (2013–2014); Amgen Foundation Summer Scholarship (2014); Prize winner at the “Lomonosov-2010” School Olympiad in Chemistry (May 2010); Prize winner at the “Lomonosov-2010” School Olympiad in Biology (April 2010); First place at the III Open Olympiad in Quantitative Biology, Moscow Institute of Physics and Technology State University (March 2010)

*Academic Mentor: Alexander Krasnitz*

**Ngoc “Tumi” Tran,** San Jose State University; B.S. in Chemistry, concentration in Biochemistry (2013); ABRCMS Poster Presentation Award (2014); SJSU President’s Scholar (2014); SJSU Robert & Eleanor Brady Scholarship (2013); SJSU Dean’s Scholar (2013); SJSU Neptune Scholarship (2012, 2013); SJSU General Endowment Scholarship (2012, 2013); Eberhart Memorial Scholarship (2011)

*Academic Mentor: Leemor Joshua-Tor*

### Academic Mentoring

The Watson School takes great pride in the mentoring that it offers its students. One example is our two-tiered mentoring approach, whereby each student chooses both an academic and a research mentor. The academic mentor is a critical advisor during the intensive coursework of the first term, during their rotations and when identifying a suitable research mentor. Furthermore, the academic mentor continues to follow them throughout their doctoral experience, often serving as important advocates for the students. Entering students select, by mutual agreement, a member of the research or nonresearch faculty to serve as an academic mentor. This program continues to
receive much support from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in our success. The following are the Academic Mentors for the Entering Class of 2015.

<table>
<thead>
<tr>
<th>STUDENT MENTOR</th>
<th>STUDENT MENTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benjamin Berube</td>
<td>Zachary Lippman</td>
</tr>
<tr>
<td>Kristina Grigaityte</td>
<td>John Inglis</td>
</tr>
<tr>
<td>Matt Lee</td>
<td>Nicholas Tonks</td>
</tr>
<tr>
<td>Katarina (Katie) Meze</td>
<td>Jay Lee</td>
</tr>
<tr>
<td>Alexandra (Ally) Nowlan</td>
<td>Sofya Polyanskaya</td>
</tr>
<tr>
<td>Jessica Tollkhun</td>
<td>Alexander Krasnitz</td>
</tr>
<tr>
<td>Alexander Krasnitz</td>
<td>Leemor Joshua-Tor</td>
</tr>
</tbody>
</table>

Recruiting Efforts

This year we once again focused primarily on targeted visits to graduate fairs and minority conferences to identify prospective students for the School. 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 multiprogram booklet, incorporating the graduate, undergraduate, and postdoctoral programs, was designed for this recruitment season. Additionally, e-mails were sent to personalized contacts and an electronic mailing list of more than 50,000 individuals who receive information from the Cold Spring Harbor Laboratory Press or have attended Meetings & Courses or Banbury meetings. We are grateful to these departments for sharing this contact list. We are pleased with the number of applications received—299—for the Entering Class of 2016, the second largest number to date.

Interinstitutional Academic Interactions

WSBS students account for approximately half of the total graduate student population here at CSHL; the other half comprise 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 fraction 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 established relationships with other institutions around the world, enabling their students to conduct research here at CSHL. Currently we have visiting students from institutions in France, India, Portugal, and the United States. The Watson School provides a contact person for the students and maintains relationships with the administrators from their home institutions. These students are fully integrated into the CSHL community and receive all the necessary assistance as they navigate the complexities of performing doctoral research away from their home institutions. The following students, listed in the box below, joined us from SBU this year.

<table>
<thead>
<tr>
<th>STUDENT</th>
<th>CSHL RESEARCH MENTOR</th>
<th>SBU PROGRAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicholas Gallo</td>
<td>Linda Van Aelst</td>
<td>Neuroscience</td>
</tr>
<tr>
<td>Yong Jin Kim</td>
<td>Adrian Krainer</td>
<td>Molecular Genetics and Microbiology</td>
</tr>
<tr>
<td>Navyateja Korimerla</td>
<td>Raffaella Sordella</td>
<td>Biomedical Engineering</td>
</tr>
<tr>
<td>Ricardo Rauldes, Jr.</td>
<td>Z. Josh Huang</td>
<td>Neuroscience</td>
</tr>
<tr>
<td>Priyadarshini Ravichandran</td>
<td>Lloyd Trotman</td>
<td>Molecular and Cellular Biology</td>
</tr>
<tr>
<td>Junyuan Song</td>
<td>Alexander Krasnitz</td>
<td>Applied Math and Statistics</td>
</tr>
<tr>
<td>Viacheslav Zhygulin</td>
<td>Alexander Krasnitz</td>
<td>Applied Math and Statistics</td>
</tr>
</tbody>
</table>
Graduate Student Symposium

Each year the students participate in two Graduate Student Symposia held at the Laboratory’s Genome Research Center in Woodbury: one in May, the other in October. Each Symposium consists of senior students giving short talks, while coffee breaks and lunch provide opportunities for more informal interactions. The prize for best talk for the May session was shared by Arkarup Bandyopadhyay (WSBS, Albeau lab) and Tobiloba Oni (SBU, Tuveson lab), and for the October session it was awarded to Justus Kebschull (WSBS, Zador lab). We are grateful to the two student chairs—Tobiloba Oni (SBU) and Paul Masset (WSBS)—for their hard work and to WSBS’s Kim Geer for helping make the Symposium a great success.

Graduate Student and Postdoctoral Fellow Departures

With each year come not only new arrivals but also departures. The following graduate students and postdoctoral fellows left the Laboratory during 2015.

Postdoctoral Fellows

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Martin Akerman</td>
<td>Bradford Elmer</td>
</tr>
<tr>
<td>Francesco Anselmi</td>
<td>Ilaria Falciatori</td>
</tr>
<tr>
<td>Rachaptra Balkunde</td>
<td>Astrid Haase</td>
</tr>
<tr>
<td>Jan Bergmann</td>
<td>Anna Hangyane Benkovics</td>
</tr>
<tr>
<td>Martin Brill</td>
<td>Toshihide Hige</td>
</tr>
<tr>
<td>Vincenzo Corbo</td>
<td>Junka Hirokawa</td>
</tr>
<tr>
<td>Kate Creasey</td>
<td>Guy Horev</td>
</tr>
<tr>
<td>Benjamin Czech</td>
<td>Yannick Jacob</td>
</tr>
<tr>
<td>Mark Dominique</td>
<td>Yinpeng Jiao</td>
</tr>
<tr>
<td>Dennis Eckmeier</td>
<td>Daniel Jones</td>
</tr>
<tr>
<td>Franchesca Anselmi</td>
<td>Yongsoo Kim</td>
</tr>
<tr>
<td>Rachaptra Balkunde</td>
<td>Stefan Klingler</td>
</tr>
<tr>
<td>Jan Bergmann</td>
<td>Seung-Jae Lee</td>
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<tr>
<td>Martin Brill</td>
<td>Christophe Liseron-Monfils</td>
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<tr>
<td>Vincenzo Corbo</td>
<td>Fatih Mercan</td>
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<td>Kate Creasey</td>
<td>Adam Mohamed Naguib</td>
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<tr>
<td>Benjamin Czech</td>
<td>Shuang Ni</td>
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<tr>
<td>Mark Dominique</td>
<td>Soonju Park</td>
</tr>
<tr>
<td>Dennis Eckmeier</td>
<td>Mario Penzo</td>
</tr>
<tr>
<td>Claire Rebbeck</td>
<td>Sachin Ranade</td>
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<tr>
<td>Leah Sabin</td>
<td></td>
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<td>Ana Santos de Almeida</td>
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<tr>
<td>Serif Senturk</td>
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<tr>
<td>Yichun Shuai</td>
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<tr>
<td>Oliver Tam</td>
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<tr>
<td>Shaoei Teng</td>
<td></td>
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<tr>
<td>Wim Verleyen</td>
<td></td>
</tr>
<tr>
<td>Chia-Lin Wang</td>
<td></td>
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<tr>
<td>Yu-Ting Yang</td>
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</tbody>
</table>

Graduate Students

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pedro Alexandre Garcia Da Silva</td>
<td>Silvia Fenoglio</td>
</tr>
<tr>
<td>Philippe Batut</td>
<td>Michael Giangrasso</td>
</tr>
<tr>
<td>Colleen Carlston</td>
<td>Wee Siong Goh</td>
</tr>
<tr>
<td>An-Yun Chang</td>
<td>Hyun-Yong Jeon</td>
</tr>
<tr>
<td>Kristen Delevich</td>
<td>Devinn Lambert</td>
</tr>
<tr>
<td>Nicolas Erard</td>
<td>Ha Yan Lee</td>
</tr>
<tr>
<td>David Nunes Raposo</td>
<td>Jia-Ray Yu</td>
</tr>
<tr>
<td>Ian Peikon</td>
<td>Cinthya Zepeda Mendoza</td>
</tr>
<tr>
<td>Mathangi Ramesh</td>
<td>Jason Tucciarone</td>
</tr>
<tr>
<td>Jason Tucciarone</td>
<td>Jack Walleshauser</td>
</tr>
<tr>
<td>Ming Yang</td>
<td>Xin Zhou</td>
</tr>
</tbody>
</table>

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, Bo Li, David Spector, David Stewart, and Linda Van Aelst. I would also like to thank the student representatives Debjani Pal (SBU) and Onyekachi Odoemene (WSBS), who contributed to discussions and provided useful suggestions and feedback from their colleagues.

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 2015 programs possible, including the Annenberg Foundation, Lita Annenberg Hazen Foundation, Bristol-Myers Squibb Corporation, 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. and Mrs. Mark Hoffman, Annette Kade Charitable Trust, Mr. David H. Koch, Mr.
and Mrs. Robert D. Lindsay and Family, Mr. and Mrs. David Luke III, Mr. and Mrs. William R. Miller, OSI Pharmaceuticals Foundation, Estate of Edward L. Palmer, Mr. and Mrs. John C. Phelan, the Quick Family, Estate of Elisabeth Sloan Livingston, the Starr Foundation, the Roy J. Zuckerberg Family Foundation, and anonymous donors.

We are also continuously grateful for our endowed lectureships: the John P. and Rita M. Cleary Visiting Lectureship, the George W. Cutting Lectureship, the William Stamps Farish Lectureship, the Martha F. Gerry Visiting Lectureship, the Edward H. Gerry Visiting Lectureship, the Edward H. and Martha F. Gerry Lectureship, the Susan T. and Charles E. Harris Visiting Lectureship, the Klingenstein Lectureship, the Mary D. Lindsay Lectureship, the Pfizer Lectureship, the George B. Rathmann Lectureship, the Seraph Foundation Visiting Lectureship, and the Sigi Ziering Lectureship.

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.

Student and Alumni Achievements

To date, 85 students have received their Ph.D. degree from the WSBS. Twenty-two graduates currently hold tenure-track faculty positions, including seven who have attained the rank of Associate Professor. In 2015, Oliver Fregso accepted an assistant professor position at UCLA, Hiroki Asari is now a Group leader at EMBL in Monterotondo, Rome, and Elizabeth (Thomas) Bartom is an Assistant Professor at Northwestern University. Our graduates have also moved into influential positions in administration, publishing, consulting, and industry. In 2015, Amy Rappaport took a scientist position at Theranos, Inc., Ian Peikon joined Kallyope as a Scientist, and Colin Malone is now Director of Genomic Analysis and Technical Operations at Columbia University Medical Center.

The WSBS students continue to impress us with their accomplishments. They publish their research findings in prestigious international journals (more than 320 papers to date), present their research at international meetings, and obtain fellowships to pursue their research interests.

In 2015, our current students and alumni were successful in receiving the following prestigious awards and fellowships:

- WSBS graduate Ian Peikon received the prestigious Harold M. Weintraub Graduate Student Award.
- Three WSBS students, Giorgia Battistoni, Laura Maiorino, and Georgi Yordanov, were awarded predoctoral fellowships from the Boehringer Ingelheim Fonds.
- WSBS graduate Amy Leung received a K01 Research Scientist Development Award from the National Institutes of Health.
- WSBS graduate Zinaida Perova received a Marie Curie Research Fellowship.
- WSBS graduate Katherine McJunkin received an NIH K99/R00 Pathway to Independence Award.
- WSBS student Robert Aboukhalil was accepted into the Insight Data Science Fellows Program.

Prizes for the best posters by a postdoctoral fellow and by a graduate student were awarded at the Laboratory’s annual In-House Symposium held in November 2015. The poster session provides a forum for the postdoctoral fellows and students to show off their research and gives the entire scientific community a chance to come together and share ideas beyond the walls of their individual laboratories. This year, Annabel Romero Hernandez, a Watson School student from Hiro Furukawa’s laboratory, won the graduate student prize. The postdoctoral prize was awarded to Sarah Diermeier from David Spector’s laboratory.
2015 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS


### WSBS GRADUATES IN FACULTY AND SENIOR POSITIONS

<table>
<thead>
<tr>
<th>Name</th>
<th>Current position</th>
</tr>
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<tbody>
<tr>
<td>Hiroki Asari</td>
<td>Group Leader, EMBL, Monerotondo, Rome</td>
</tr>
<tr>
<td>Elizabeth (Thomas) Bartom</td>
<td>Assistant Professor, Northwestern University, Illinois</td>
</tr>
<tr>
<td>Mitchell Bekritsky</td>
<td>Bioinformatics Scientist, Illumina, Cambridge, United Kingdom</td>
</tr>
<tr>
<td>Rebecca Bish-Cornelissen</td>
<td>Senior Scientific Editor, D.E. Shaw Research, New York</td>
</tr>
<tr>
<td>François Bolduc</td>
<td>Assistant Professor, University of Alberta, Canada</td>
</tr>
<tr>
<td>Darren Burgess</td>
<td>Senior Editor, <em>Nature Reviews Genetics</em>, United Kingdom</td>
</tr>
<tr>
<td>Amy Caudy</td>
<td>Assistant Professor, University of Toronto, Canada</td>
</tr>
<tr>
<td>Daniel Chitwood</td>
<td>Assistant Professor, Donald Danforth Plant Science Center, St. Louis, Missouri</td>
</tr>
<tr>
<td>Michelle Gila</td>
<td>Research Molecular Biologist, US Dept, Agriculture, The Boyce Thompson Institute; Adjunct Assistant Professor, Cornell University</td>
</tr>
<tr>
<td>Catherine Cormier</td>
<td>Program Manager, The Biobank Core Facility, St. Joseph’s Hospital and Medical Center, Arizona</td>
</tr>
<tr>
<td>Monica Dus</td>
<td>Assistant Professor, University of Michigan</td>
</tr>
<tr>
<td>Yaniv Erlich</td>
<td>Assistant Professor, New York Genome Center, Columbia University, New York</td>
</tr>
<tr>
<td>Rebecca Ewald</td>
<td>Manager, Business Development, Ventana Medical Systems, Arizona</td>
</tr>
<tr>
<td>Elena Ezhkova</td>
<td>Assistant Professor, Mount Sinai School of Medicine, New York</td>
</tr>
<tr>
<td>Patrick Finigan</td>
<td>Associate, Regulatory Affairs, Genzyme, New Jersey</td>
</tr>
<tr>
<td>Paloma Guzzardo</td>
<td>Research Group Leader, Horizon Genomics, Austria</td>
</tr>
<tr>
<td>Ira Hall</td>
<td>Associate Professor, Washington University, St. Louis, Missouri</td>
</tr>
<tr>
<td>Christopher Harvey</td>
<td>Assistant Professor, Harvard University, Massachusetts</td>
</tr>
<tr>
<td>Tomáš Hromádka</td>
<td>Assistant Professor, Slovak Academy of Sciences, Slovakia</td>
</tr>
<tr>
<td>Keisha John</td>
<td>Director, Diversity Programs, University of Virginia</td>
</tr>
<tr>
<td>Zachary Lippman</td>
<td>Associate Professor, Cold Spring Harbor Laboratory, New York</td>
</tr>
<tr>
<td>Colin Malone</td>
<td>Director of Genomic Analysis and Technical Operations, Columbia University Medical Center</td>
</tr>
<tr>
<td>Marco Mangone</td>
<td>Assistant Professor, Arizona State University</td>
</tr>
<tr>
<td>Masafumi Muratani</td>
<td>Associate Professor, University of Tsukuba, Japan</td>
</tr>
<tr>
<td>Elizabeth Murchison</td>
<td>Reader, Cambridge University; Fellow, Kings College, United Kingdom</td>
</tr>
<tr>
<td>Patrick Paddison</td>
<td>Associate Member, Fred Hutchinson Cancer Research Center</td>
</tr>
<tr>
<td>Ian Peikon</td>
<td>Scientist, Kallyope</td>
</tr>
<tr>
<td>Maria Pineda</td>
<td>Co-founder and CEO, Envisagenics, Inc., New York</td>
</tr>
<tr>
<td>Amy Rappaport</td>
<td>Assay Development Scientist, Theranos, California</td>
</tr>
<tr>
<td>Emiliano Rial-Verde</td>
<td>Director, Corporate Development, Bunge Limited, New York</td>
</tr>
<tr>
<td>Frederick Rollins</td>
<td>Consultant, L.E.K. Consulting, Massachussets</td>
</tr>
<tr>
<td>Felix Schlesinger</td>
<td>Bioinformatics Scientist, Illumina, California</td>
</tr>
<tr>
<td>Ji-Joon Song</td>
<td>Associate Professor, Korea Advanced Institute of Science and Technology (KAIST), South Korea</td>
</tr>
<tr>
<td>Niraj Tolia</td>
<td>Associate Professor, School of Medicine, Washington University, St. Louis, Missouri</td>
</tr>
<tr>
<td>Wei Wei</td>
<td>Assistant Professor, University of Chicago, Illinois</td>
</tr>
<tr>
<td>Susann Weissmueller</td>
<td>Equity Research Associate, Roth Capital Partners</td>
</tr>
<tr>
<td>Jeremy Wilusz</td>
<td>Assistant Professor, University of Pennsylvania</td>
</tr>
</tbody>
</table>

*Alexander Gann*

*WSBS Professor and Dean*
SPRING CURRICULUM

Topics in Biology

ARRANGED BY Alyson Kass-Eisler and Jan A. Witkowski

Each year, invited instructors offer 7-day courses at the Banbury Conference Center exploring specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning and evening lectures, as well as afternoon sessions during which students read assigned papers or work on problem sets and presentations. In Spring 2015 there were two such courses: Physical Biology of the Cell and Evolution.

Physical Biology of the Cell

March 1–March 7

INSTRUCTOR

Rob Phillips, Caltech

VISITING LECTURERS

Joe Blitzstein, Harvard University
Patricia Bassereau, Institut Curie
Jané Kondev, Brandeis University
Hernan Garcia, University of California, Berkeley
Sanjoy Mahajan, Massachusetts Institute of Technology
Aleksandra Walczak, Ecole Normale Supérieure

TEACHING FELLOWS

Griffin Chure, Caltech

(From left to right) Georgi Yordanov, Giorgia Battistoni, Brinda Alagesan, Aleksandra Walczak, Abram Santana, Sanchari Ghosh, Maria Nattestad, Patricia Bassereau, Ray Ho, Talitha Forcier, Tyler Garvin, Annabel Romero Hernandez, Rob Phillips, Griffin Chure, Paul Masset, Lital Chartarisky, Hernan Garcia, Michael Guthrod, Laura Maiorino, Trevor Grand Pre, Armando Reimer
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. They began with “order of magnitude biology,” showing how simple estimates can be exploited in biology. They showed how to construct simple models of a variety of different biological problems, primarily using the tools of statistical mechanics. One of the key themes of the course was to show how physical biology unites and organizes topics in a fundamentally different way, often revealing that topics that are nearby in physical biology seem unrelated when viewed from the vantage point of molecular or cell biology. The instructors guided the students from start to finish on several modeling case studies.

Evolution

March 22–March 28

Attended by the entering classes of 2011 and 2014

INSTRUCTOR

Nipam Patel, University of California, Berkeley

VISITING LECTURERS

Nick Pyenson, Smithsonian Institution
Rob DeSalle, American Museum of Natural History
Mansi Srivastava, Massachusetts Institute of Technology

TEACHING FELLOWS

Kelley Harris, University of California, Berkeley
Jenna Judge, University of California, Berkeley

The field of evolutionary biology touches upon all other areas of the biological sciences, because every form of life and every biological process represent an ongoing evolutionary “experiment.” Our
aim in this course was to both discuss our understanding of the mechanisms of evolution and explore how evolutionary data can be used to further our understanding of various biological problems.

The course began with a discussion of the diversity of organisms that currently exist and methods for understanding the evolutionary relationships between these organisms. The students went on to study how paleontological data is collected and used to understand the history of life on Earth and examined how DNA sequence data can be used to understand the evolutionary history of organisms, genes, and genomes. Within this molecular and genetic framework, the focus shifted to the mechanisms of evolutionary change and how variation within populations leads to the evolution of new species. Finally, there was discussion of how morphological changes are brought about through evolutionary changes in development and gene regulation. The hope was that this course would provide the student with a general overview of evolutionary biology and ways of using evolutionary data to gain further insight into all manner of biological problems. The course included a class favorite field trip and curator tour at the American Museum of Natural History in New York City.

Special Course: Optical Methods

January 6–11
Attended by the entering class of 2014

INSTRUCTORS
Florin Albeanu, CSHL
Adam Kampff, Champalimaud Center, Portugal

VISITING LECTURERS
Florian Engert, Harvard University
Georg Keller, Friedrich Miescher Institute, Switzerland

GUEST LECTURERS
Mikala Egeblad, CSHL
Pavel Osten, CSHL
David Spector, CSHL

TEACHING FELLOWS
Francesca Anselmi, CSHL
Priyanka Gupta, CSHL
Arkarup Banerjee, CSHL

Optical imaging techniques are widely used in all areas of modern biological research. Our aim for this course was to give students an introduction into widely used basic and advanced optical methods. Given the experimental nature of the topic, a central aim of the course was to offer students a practical hands-on experience. This included both the use of commercially available systems and, more importantly, a primer on custom building and adapting optical setups to address specific biological needs.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR
David A. Micklos

INSTRUCTORS
Amanda McBrien (lead)
Katie McAuley
Elna Gottlieb
Erin McKechnie
Bruce Nash
Brooke Roeper
Shreya Shah

As science plays an increasing role in society, there is also an increasing need for biologists to educate nonscientists of all ages about biology. The Watson School of Biological Sciences doctoral
program offers its students the opportunity to teach in the Laboratory’s Dolan DNA Learning Center, where students teach laboratory courses to high school and middle school students. In so doing, they learn how to communicate with nonbiologists and to inspire and educate creative young minds. The teaching module entailed pairs of students teaching one morning or afternoon a week for 12 weeks. In the initial weeks, the Dolan DNA Learning Center instructors taught the Watson School students the didactic process; it was not until the fifth week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research. After the fall term courses, students participate in laboratory rotations; these 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 practice giving a scientific presentation. This year, 18 WSBS faculty members served as rotation mentors, some mentoring more than one student.

<table>
<thead>
<tr>
<th>ROTATION MENTORS</th>
<th>Florin Albeanu</th>
<th>Adam Kepecs</th>
<th>Steve Shea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anne Churchland</td>
<td>Alex Koulakov</td>
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<td>Adam Siepel</td>
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<tr>
<td>Mikala Egeblad</td>
<td>Jay Lee</td>
<td></td>
<td>Raffaella Sordella</td>
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<tr>
<td>Hiro Furukawa</td>
<td>Bo Li</td>
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<td>David Spector</td>
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<tr>
<td>Molly Hammell</td>
<td>Zachary Lippman</td>
<td></td>
<td>Chris Vakoc</td>
</tr>
<tr>
<td>Leemor Joshua-Tor</td>
<td>Michael Schatz</td>
<td></td>
<td>Anthony Zador</td>
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</table>
FALL CURRICULUM

The students started the semester by attending boot camps in Molecular and Cellular Biology and in Quantitative Biology as an introduction to the techniques and terminology that they would encounter in subsequent courses. The Molecular and Cellular Biology boot camp featured seven lectures from faculty members Hiro Furukawa, Dick McCombie, Pavel Osten, David Spector, Linda Van Aelst, and Lingbo Zhang, and the Quantitative Biology boot camp lectures were given by Mickey Atwal and Justin Kinney.

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

INSTRUCTORS
- Linda Van Aelst (Lead)
- Alexander Gann
- Christopher Hammell
- Leemor Joshua-Tor
- Bo Li

GUEST LECTURERS
- Hiro Furukawa
- Justin Kinney
- Adrian Krainer
- Robert Martienssen
- Christopher Vakoc

In this core course, which forms the heart of the curriculum, students (1) acquired a broad base of knowledge about the biological sciences, (2) learned the scientific method, and (3) learned how to think critically. The initial four to five modules are on a different general theme; in each, students read an assigned set of research articles and at the end of the module provided written answers to a problem set that guided them through several of the articles.

Twice weekly, students attended lectures related to the module’s topic that included concepts and fundamental information as well as experimental methods. The students met among themselves to discuss the assigned papers not covered by the problem set. Each week, students spent an evening discussing the assigned articles with faculty. In the final module of the course, students participated in a mock study section in which real National Institutes of Health R01 grants were reviewed and critiqued. This allowed students to learn 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.

In 2015, the following were the module topics for this course.

<table>
<thead>
<tr>
<th>Topic</th>
<th>Instructor(s)</th>
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<tbody>
<tr>
<td>Gene Expression</td>
<td>Alex Gann</td>
</tr>
<tr>
<td>Gene Regulatory Logic and the Construction of Multicellular Organisms: Insights from Flies, Plants, and Worms</td>
<td>Christopher Hammell</td>
</tr>
<tr>
<td>The Brain: Wiring, Plasticity, and Maladaptation</td>
<td>Bo Li</td>
</tr>
<tr>
<td>Macromolecular Structure and Function</td>
<td>Leemor Joshua-Tor</td>
</tr>
<tr>
<td>Study Section</td>
<td>Linda Van Aelst</td>
</tr>
</tbody>
</table>
The Darrell Core Course on Scientific Exposition and Ethics

The 2015 Scientific Exposition and Ethics (SEE) core course was led by David Stewart, Arne Stenlund, and Mikala Egeblad. 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.

INSTRUCTORS
David J. Stewart (Lead)
Carrie Cowan
Mikala Egeblad
Arne Stenlund

GUEST LECTURERS
Lisa Bianco
Carrie Cowan
Diane Esposito
Alyson Kass-Eisler
Stephanie Woods

VISITING LECTURERS
Olga Akselrod, The Innocence Project
Victoria Aranda, Nature Medicine
Elizabeth Bass, Alan Alda Center for Communicating Science
Michael Bielski, The Center for Biotechnology
Keith Baggerly, M.D. Anderson Cancer Center
Robert Charrow, Greenberg Traurig, LLC
Stephen Hall, New York University
Avner Hershlag, North Shore University Hospital
Yvette Seger, FASEB
Tung-Tien Sun, New York University

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 2014 took a total of four Specialized Disciplines courses this fall: Cancer, Quantitative Biology, Systems Neuroscience, and Genetics and Genomics.

Cancer

September 8–18

INSTRUCTORS
David Tuveson (Lead)

GUEST LECTURERS
Mikala Egeblad
Douglas Fearon
Raffaella Sordella
David Spector
Bruce Stillman
Christopher Vakoc

Cancer represents an increasing cause of morbidity and mortality throughout the world as health advances continue to extend the life spans of our populations. Although our basic understanding of cancer has increased considerably since 1971, when then-U.S. President Richard Nixon initiated the “War on Cancer,” our ability to translate this knowledge into a health benefit for patients has been restricted to certain malignancies and often only temporarily. Importantly, specific hypotheses developed from our knowledge of cancer biology can be tested in increasingly complex model systems ranging from cell culture to genetically engineered mouse models, and such investigations should prove invaluable in discovering new methodologies for the detection, management, and treatment of cancer in humans.

At the conclusion of this course, students were able to elaborate an understanding of cancer as a pathobiological process that invades our bodies without offering any known benefit to the host, discuss how we diagnose cancer today, and contemplate how to replace the methods currently used to treat cancer. Students were also able to design tractable methods to investigate fundamental aspects of cancer biology and are familiar with translational approaches to defeating cancer. Topics covered in this course included biochemistry, epigenetics, immunology, resistance, growth control, microenvironment, noncoding RNA, and disease modeling. The implications of the biological findings for cancer prevention, diagnosis, and treatment were covered.

Quantitative Biology

August 26 and 27, October 5–27, November 12–December 1

INSTRUCTORS
Mickey Atwal (Lead)
Adam Siepel

GUEST LECTURERS
Justin Kinney (CSHL Fellow)
Alexander Krasnitz

Quantitative reasoning is a powerful tool for uncovering and characterizing biological principles, ranging from the molecular scale all the way to the ecological. With the advent of high-throughput
technologies in genomics and neuroscience it has become increasingly necessary for biological researchers to be able to analyze and interpret high-dimensional data and frame biological hypotheses mathematically. To this end, this course aimed to equip the students with a basic training in computer programming, modern statistical methods, and physical biology. By the end of the course students were able to not only answer many of the statistical questions that arise in data analyses but were also familiar with the more complex techniques employed by fellow computational biologists. Topics covered included probabilities, statistical fluctuations, Bayesian inference, significance testing, fluctuations, diffusion, information theory, neural signal processing, dimensional reduction, Monte Carlo methods, population genetics, and DNA sequence analyses. A common theme throughout the course was the use of probabilistic and Bayesian approaches.

Systems Neuroscience

October 2–29

INSTRUCTORS
Adam Kepecs (Lead)
Stephen Shea

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 and 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 students then examined classical conditioning and asked how changes in synaptic transmission could underlie such behavior. Associative learning computational models of the learning process were also 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 conclud ed with a discussion of the role of dopamine in reward and learning, the theory of reinforcement learning, and pathologies of reward-seeking behavior.

Genetics and Genomics

November 2–17

INSTRUCTORS
Zachary Lippman (Lead)
Adam Siepel

GUEST LECTURER
David Jackson

VISITING LECTURER
Daniel Grimanelli, IRD, Montpellier, France
Guillaume Lettre, University of Montreal

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 placed 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.
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 2015, new members Dhananjay Huilgol, Grinu Matthew, and Hillary Schiff joined returning members Sara Ballouz and Saikat Nandi. The PDLC organized a successful retreat this year aimed at fostering networking and collaboration. The retreat took place at the Banbury Conference Center and included sessions on Going on the Job Market—Faculty Search Committee Perspective, Funding and Grantsmanship, Opportunities in Biotech/Pharma, Start-ups and Tech Transfer, Opportunities in Publishing & Science Writing, and a Mock Chalk Talk. The PDLC also oversees and distributes funds to two career development groups as described below. These groups are primarily composed of postdoctoral fellows but 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.

Career Development Program (CDP) is interested in providing programming geared toward careers in academia. CDP continued the successful “Conversations with Faculty” series in which the postdocs were given career insights in an informal and interpersonal format. Sessions included Going on the Job Market—the Applicant Perspective, with guest panelists Olga Anczukow-Camarda and Jay Lee; Obtaining a Faculty Position in the United Kingdom, given by Watson School of Biological Sciences alumnus Elizabeth Murchison; and Starting Your Own Lab, with Jay Lee, Molly Hammell, and Jessica Tollkuhn. CDP also organized five Mock Chalk Talks for those postdocs who are preparing for a job interview in academia.

We have also been working on building connections with local colleges and universities to provide teaching experience opportunities for interested postdocs. Informal partnerships are now in place with Molloy College and Long Island University, and we are working with Adelphi
Postdoctoral Program

University to expand these opportunities. In addition, selected postdoctoral fellows continue to participate in the Watson School as tutors either one-on-one or in the classroom setting, thus providing the postdocs with valuable experience in teaching and mentoring. One of the postdocs who taught a course at Long Island University this fall helped prepare by attending the Scientists Teaching Science course held at the New York Academy of Sciences. She presented what she learned in a CDP session focused on teaching.

The Bioscience Enterprise Club (BEC) is most interested in providing information for nonacademic careers, and it has 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 BEC hosted visits from the publishing company eLife and from Enzo Life Sciences. They also hosted a talk by Dr. Jennifer Doudna, a pioneer in CRISPR–Cas9 technology. Dr. Doudna is a co-founder of Caribou Biosciences (http://cariboubio.com/). In addition, they hosted two workshops in computer science. The first was an intro to Galaxy + RNA-Seq and the second was on making figures and plots in R.

Once again, BEC organized a symposium for postdocs at the Laboratory and the greater New York area called Beyond the Bench, highlighting career opportunities outside academia. Featured speakers included Dr. Rich Roberts, Nobel Laureate & CSO at New England Biolabs; Dr. Hillary Sussman, Executive Editor at Genome Research; Dr. Chris Wiggins, Chief Data Scientist at the New York Times; Dr. Nicholas Gale, Senior Director at Regeneron Pharmaceuticals; and Dr. Uli Bialucha, Group Leader at Novartis.

To celebrate National Postdoc Appreciation week, a volleyball game and barbeque were held on campus. They provided a great opportunity for the community to get together for some fun but also to network and find out about the ongoing programs we have been running.

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 CSHL community as a whole. This year, the postdoctoral prize was awarded to Sarah Diermeier from David Spector’s laboratory.

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 Perspectives in STEM, an evening with Dr. Cherry Murray; Making the Leap: A Nonacademic Career Planning and Job Search Boot Camp; Scientists Teaching Science; Personal Branding; Ph.D. & Postdoc Career Symposium, at the New York Bio Conference; From Scientist to CSO: Experiencing the Scientific Method as Your Guide to Career Success; Grantsmanship for Postdocs; Policies That Influence Gender Balance in STEM; Nontraditional Postdocs; Info Session about the NSF Graduate Research Fellowship Program; The Business of STEM: Start-ups, Technologies, Entrepreneurship, and Management; Interviewing Etiquette for STEM Professionals; and Success Stories in Nonacademic Career Tracks: Overcoming the Barriers of an International Scientist in the USA. They also hosted What Can You Be With a PhD?, a science and technology–focused career convention, which provides a unique opportunity to
connect the private and public sector, industry, and academe with the rising generation of scientists and engineers who will become tomorrow’s leaders and innovators.

Since 2011, the Laboratory has held a Cancer Gene Discovery and Cancer Biology Postdoctoral Research Training Program with funding from the National Cancer Institute and National Institutes of Health (NIH). The program provides fellows with training in four integrated research areas: Cancer Genetics, Cancer Regulation and Cell Proliferation, Signal Transduction, and Quantitative Biology. This summer we submitted a competitive renewal of this funding to the NIH. The grant received positive reviews and we are hopeful that the grant will be renewed in 2016.

Finally, an important measure of our postdoctoral program’s success is the ability of postdoctoral fellows to secure positions at the end of their training. In 2015, our fellows took positions at ARUP Laboratories, Audible Inc., Envisagenics Inc., Cell Motion Laboratories, Howard University, Izmir International Biomedicine & Genome Institute, Memorial Sloan Kettering Cancer Center, Mirimumus Inc., the National Institutes of Health, New York Genome Center, Penn State University, Regeneron, Technion—Israel Institute of Technology, Transcriptic, USDA-ARS, and Yale University.
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. To ensure a smooth transition into the Laboratory community and research, during the first week the students attend various orientations and receive a guided historical tour of campus and all the facilities and resources available to them. The URP attendees 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 in formal and informal activities. Some of the 2015 activities included a pizza party with Dr. and Mrs. Watson, dinner with Dr. and Mrs. Stillman, volleyball games, designing the URP t-shirt, competing in the annual Scavenger Hunt, and the ever-famous URP versus PI volleyball match.

The students’ scientific development is the most important component of the program. Therefore, in the beginning of the summer, each URP attendee writes an abstract and presents a talk on his or her proposed research. The URP participants work alongside scientists and become increasingly independent throughout the summer. Concluding the program in August, each URP student prepares a final report and presents his or her results in a 15-minute talk at the URP Symposium. As in previous years, the program directors and faculty mentors were highly impressed with the accomplishments of the URP students.
The following 20 students, selected from 1047 applicants, took part in the 2015 program:

**Melanie Abegglen**  
Advisor: Robert Martienssen  
Funding: James D. Watson Undergraduate Scholar  
DNA Methylation and Epigenetic Inheritance in *Arabidopsis thaliana*

**Katrina Haught**  
Advisor: Leemor Joshua-Tor  
Funding: Howard Hughes Medical Institute Scholar  
Characterization of Human Argonaute Motifs at the N-Terminal of GW182

**Marley Alford**  
Advisor: Michael Schatz  
Funding: National Science Foundation Scholar  
Threading through the Breast Cancer Genome with PacBio Sequencing Data

**Jasmine Johnson**  
Advisor: Gholson Lyon  
Funding: Libby Fellowship  
Differential Analysis of RNA-Seq Data in Ogden Syndrome

**Patrick Aoude**  
Advisor: Christopher Hammell  
Funding: James D. Watson Fellow  
Posttranscriptional Gene Regulation in *Caenorhabditis elegans* by the K Homology (KH) Domain Protein, LIN-67

**Edith Jones**  
Advisor: Christopher Vakoc  
Funding: William Townsend Porter Foundation Scholar  
Exploring the Requirement of TAFs 9/10/12 in Different Genetic Backgrounds of Acute Myeloid Leukemia

**Bobby Baraldi**  
Advisor: Jesse Gillis  
Funding: H. Bentley Glass Fellowship  
Computational Analysis of Noncoding RNA Coexpression

**Samuel Kovaka**  
Advisor: Thomas Gingeras  
Funding: Former URP Fund Scholar  
Characterization of Isoforms in Long-Read RNA-Seq Data Sets

**Ethan Baker**  
Advisor: W. Richard McCombie  
Funding: William Shakespeare Fellowship  
Comparative Analysis of PacBio Libraries Reveals Nonstochastic Biases in Sites of DNA Nicking

**Sally Li**  
Advisor: Z. Josh Huang  
Funding: Dorcas Cummings Scholar  
Chandelier Cell Selectivity in a Prefrontal Fear Circuit

**Kevin Chu**  
Advisor: Josh Dubnau  
Funding: Vannevar Bush 125th Anniversary Fellow  
The Transposon Storm Hypothesis of Neurodegeneration

**Gabriel Mel**  
Advisor: Partha Mitra  
Funding: Joan Redmond Read Fellowship  
Algorithms for Automatic Anatomical Segmentation in Mouse Brain Nissl Slices

**Amanda Cruz**  
Advisor: Alea Mills  
Funding: Garfield Fellowship  
Chd5 Epigenetically Regulates the Genes That Underlie Tumor Heterogeneity in Glioma

**Jordan Ontiveros**  
Advisor: Zachary Lippman  
Funding: Emanuel Ax Fellowship  
The Tomato Flowering Transition Proteins TMF and BOP Enhance Transcription In Vitro

**Charlotte Darby**  
Advisor: Doreen Ware  
Funding: 30th Anniversary URP Scholar  
Conservation of Transcriptional Regulation in microRNA-mediated Stress Responses between Maize and *Arabidopsis*

**Luqun Shen**  
Advisor: Lloyd Trotman  
Funding: University of Notre Dame URP Scholar  
Mitochondrial DNA and Bone Metastasis in Prostate Cancer

**Michelle David**  
Advisor: Anne Churchland  
Funding: Von Stade Fellowship  
Mapping Visual Areas in the Rat Cortex with Intrinsic Optical Imaging

**Cole Townsend**  
Advisor: Mikala Egeblad  
Funding: Robert H.P. Olney Fellow  
The Tumor Microenvironment and Phenotypic Plasticity

**Christine Gao**  
Advisor: Bruce Stillman  
Funding: Alfred L. Goldberg Fellowship  
Interactions of the Mcm3 C-Terminus and Its Homologs during Activation of the Eukaryotic Replicative Helicase

**Kellie Wilson**  
Advisor: Florin Albeau  
Funding: Burroughs Wellcome Fellowship  
Light-Induced Olfactory Detection
PARTNERS FOR THE FUTURE

PROGRAM DIRECTOR  David Jackson
PROGRAM ADMINISTRATOR  Marygrace Navarra

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 for the program are paired with a scientist mentor and spend a minimum of 10 hours per week, September through March of their senior year, conducting original research. At the conclusion, the students present their projects to an enthusiastic audience of the students, scientific mentors and colleagues, CSHL administrators, parents, and teachers. Although the students learn a great deal about modern biology and state-of-the-art research techniques, the main advantage of the program is that they are exposed to day-to-day life in a lab. Interacting with scientists and support staff, the students learn to define and pursue a research goal while solving problems that may occur along the way.

The 2015–2016 Partners for the Future were chosen from among 53 nominations. They are:

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<tr>
<th>Partner</th>
<th>High School</th>
<th>Laboratory</th>
<th>Mentor</th>
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<tbody>
<tr>
<td>Tamanna Bhatia</td>
<td>Cold Spring Harbor High School</td>
<td>Alea Mills</td>
<td>Alea Mills</td>
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<td>Joseph Boroda</td>
<td>Massapequa High School</td>
<td>Raffaella Sordella</td>
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<tr>
<td>Cailey Brogan</td>
<td>Cold Spring Harbor High School</td>
<td>Adam Siepel</td>
<td>Noah Dukler</td>
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<td>Dana Galgano</td>
<td>Oyster Bay High School</td>
<td>Doreen Ware</td>
<td>Lifang Zhang</td>
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<td>Hanna Hong</td>
<td>Syosset High School</td>
<td>David Jackson</td>
<td>Hannes Claeyens</td>
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<tr>
<td>Trevor R. Jones</td>
<td>Harborfields High School</td>
<td>Lloyd Trotman</td>
<td>Kaitlin Watrud</td>
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Partners for the Future participants
Partner   High School   Laboratory   Mentor
Scott Kriesberg   Friends Academy   Adam Kepecs   Adam Kepecs
Sarah Lee   Syosset High School   Mikala Egeblad   Juwon Park
Suraj Muralidharan   Farmingdale High School   Robert Martienssen   Sonali Bhattacharjee
Sabrina Qi   Harborfields High School   Adrian Krainer   Adrian Krainer
Kristin Schmidt   Division Avenue High School   David Jackson   Fang Xu
Christopher Tapia   Eastport S. Manor Jr./Sr. High School   Gholson Lyon   Gholson Lyon
Julian Ubriaco   Kings Park High School   David Tuveson   David Tuveson
Vanessa Yu   Oyster Bay High School   Adam Kepecs   Hyun Jae Pi
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 Meetings & Courses program at the Laboratory attracted strong attendance in 2015, with more than 7500 meeting participants and more than 1400 course participants (trainees, teaching, and support faculty). The Cold Spring Harbor Asia program included 17 conferences and attracted about 3000 participants, bringing the anticipated year-end total for both United States- and China-based programs to more than 12,000. A major new development for the meetings program was the June opening of the beautiful new home for scientific posters, the Nicholls-Biondi Hall, which has already proved its worth many times over for the thousands of young scientists who discussed and presented their latest work in poster form.

The Laboratory held 30 academic meetings this year, which brought together scientists from around the world to discuss their latest research. The spring meeting season culminated in the 80th Cold Spring Harbor Symposium, which addressed 21st Century Genetics: Genes at Work, in celebration of the 150th anniversary of Gregor Mendel’s landmark 1865 presentation of his paper “Experiments on Plant Hybridization,” which laid the groundwork for modern genetics. The Symposium provided a current synthesis of genetic mechanisms and genome and chromosome biology and included more than 60 outstanding speakers in 12 oral sessions. The Symposium attracted almost 350 participants, including notable scientists such as Angelika Amon, Jef Boeke, Emmanuelle Charpentier, Titia de Lange, Job Dekker, Jennifer Doudna, Denis Duboule, Ronald Evans, Susan Gasser, Rudolf Jaenisch, David Kingsley, Arnold Levine, Michael Levine, Susan Lindquist, Dan Littman, Thomas Maniatis, Richard Morimoto, Svante Pääbo, David Page, Robert Tjian, Didier Trono, and Richard Young, to name but a few. Dissemination includes the proceedings of the Symposium published each year by the CSHL Press and videotaped interviews with leading speakers by editors and journalists attending the Symposium now available on our Leading Strand YouTube. The Symposium therefore reaches a much wider audience nationally and internationally than can possibly attend.

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. 2015 saw the introduction of several successful new meetings on Biology and Genomics of Social Insects, Fundamental Immunology and Its Therapeutic Potential, and Probabilistic Modeling in Genomics, while the new conference on Genome Engineering: The CRISPR-Cas Revolution unsurprisingly attracted a packed house. Many of these meetings are essential for those in the field and are held on a biennial basis. Continuing the CSHL Genentech Center Conferences on the History of Molecular Biology and Biotechnology, the 2015 conference addressed The Evolution of Sequencing Technology: A Half-Century of Progress. Partial support for individual meetings is provided by grants from the National Institutes of Health (NIH), the National Science Foundation (NSF), other foundations, and companies. Core support for the meetings program is provided by the Laboratory’s Corporate Sponsor Program.

The Courses program covers a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. Instructors update their course curricula annually, invite new speakers who bring a fresh perspective, and introduce new techniques and experimental approaches based on student feedback and progress in the field. Recently introduced courses on Single Cell Analysis and Synthetic Biology run in parallel with well-established courses in genetics,
neuroscience, plant biology, and bioinformatics. New techniques—for example, genome editing using tools such as CRISPR—are introduced as methodologies develop and evolve. We strongly encourage each course to include the latest technical and conceptual developments in their respective fields. Instructors, lecturers, and assistants come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor. Students include advanced graduate students, postdoctoral trainees, and principal investigators and senior scientists from around the world.

A new workshop on Neural Data Analysis was held at the Banbury Conference Center. Today’s technologies enable neuroscientists to gather data in quantities previously unimagined, with computational resources and new analysis methods proliferating at a rapid pace. This course was designed to help neuroscience practitioners develop conceptual and practical capabilities to meet the challenges posed by the analysis of large functional data sets. A new 4-day Scientific Writing Retreat was also added in 2015 as part of a small series of workshops in professional development that are now supported by the National Institute of General Medical Sciences. Guidance in small peer groups was offered on how to improve the clarity and effectiveness of an individual’s professional writing in the development of manuscripts, grant proposals, job applications, and/or research/teaching statements.

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 remarkably well. We would especially like to thank Drs. Stephan Brenowitz, James Eberwine, Daniel Geschwind, Karmella Haynes, Darmaid Hughes, Mark Johnson, Paul Kammermeier, Beth Lazazzera, James Love, Julius Lucks, Gabor Marth, Pamela Peralta-Yahya, Shin-Han Shiu, Sarah Spence, Jack Waters, Fitnat Yildiz, and Karen Zito—whose exemplary teaching and leadership of their respective courses have benefited so many young scientists.

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. Major new funding from the Helmsley Charitable Trust and the National Institute of General Medical Sciences had a significant impact on the courses program. In particular, a new program, the Helmsley Interdisciplinary Fellowship Fund, provided significant fellowships for course trainees with formal scientific training outside of biology and scholarships for those individuals making major transitions within biology. The courses are further supported by multiple awards from the NIH and the 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 6th 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 in the Suzhou Innovation Park high-technology suburb. In 2015, 17 scientific conferences and two special CSHA sessions at Japanese meetings were held, predominantly attracting scientists from the Asia/Pacific region. CSHA’s scientific program includes large symposia and meetings, training workshops, and Banbury-style discussion meetings. A special meeting on International Brain Projects was held in Suzhou in June, and the recommendations of this conference were published in the October 2 issue of Science. This program is described in more detail in a separate Annual Report.

The meetings and courses program staff is composed of 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. 2015 saw the rollout of a revamped website that now
integrates seamlessly with our back-end database. We saw the departure of Demetria Mudar, one of the Conference Coordinators, to sunnier climes, and welcomed a number of new staff in 2015, including Rachel Lopez as Office and Operations Manager, Alicia Franco as Assistant Registrar, Evaluation & Impact, and Matthew Denninger as an additional Programmer.

Terri Grodzicker
Dean of Academic Affairs

David Stewart
Executive Director,
Meetings and Courses Program/
President, Cold Spring Harbor Asia
The year 2015 marked the sesquicentennial anniversary of Gregor Mendel’s landmark 1865 presentation of his paper “Experiments on Plant Hybridization,” which laid the groundwork for modern genetics. Seminal discoveries throughout the 20th century followed, not least of which was the demonstration that chromatin carried traits, the formalization of the concept of the gene as the hereditary unit, the discovery of DNA as the hereditary material, the discovery of the double-helix structure of DNA, and the elucidation of many mechanisms now known to operate to express and protect the hereditary material in its nuclear, cellular, and organismal context. The year also marked the 125th anniversary of Cold Spring Harbor Laboratory, as well as the 80th Cold Spring Harbor Laboratory Symposium on Quantitative Biology, in this preeminent and storied series of landmark meetings initiated by Reginald Davenport in 1933. It therefore seemed fitting to focus this year’s Symposium on 21st Century Genetics: Genes at Work to provide a current synthesis of genetic mechanisms and genome/chromosome biology. The decision to plan the 2015 Symposium on this theme reflects the enormous research progress achieved in recent years and was intended to provide a broad synthesis of the current state of the field, setting the stage for future discoveries and application.

Opening night speakers included Robert Tjian (HHMI/University of California, Berkeley), who addressed probing transcription regulation by single-molecule imaging; Denis Duboule
(EPF Lausanne, Switzerland), who spoke about long-range regulation during development and evolution; Ron Evans (Salk Institute for Biological Studies), who talked about nuclear receptors—feast, famine, and physiology; and Angelika Amon (Massachusetts Institute of Technology), who addressed the effects of aneuploidy on cellular fitness and tumorigenesis. Svante Pääbo (Max-Planck Institute for Evolutionary Anthropology, Germany) addressed “The Genetic Legacy of Neanderthals” in an outstanding Dorcas Cummings Lecture for Laboratory friends, neighbors, and Symposium participants in advance of the annual dinner parties. Susan Gasser delivered a masterful Summary at the conclusion of the Symposium immediately prior to the final banquet.

This Symposium was attended by almost 350 scientists from U.S. and international universities, and the program included 65 invited presentations and 135 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. A collection of interviews by Rhiannon Macrae (Cell Press), Guy Riddihough (Science Magazine), Richard Sever (CSHL Press), Jiaying Tan (Cell Press), Jan Witkowski (CSHL Banbury Center), and Eytan Zlotorynski (Nature Reviews Molecular Cell Biology) with leading experts in the field were arranged during the Symposium and distributed as free video from the Cold Spring Harbor Symposium interviews web site.

Contributions from the following companies and foundations provide core support for the Symposium and the Cold Spring Harbor meetings program: Agilent Technologies, Bristol-Myers Squibb Company, Genentech, Life Technologies (part of Thermo Fisher Scientific), Monsanto Company, and New England BioLabs. 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

Gene Control
Chairperson: K. Zaret, University of Pennsylvania Perelman School of Medicine, Philadelphia

Chromatin Domains
Chairperson: G. Almouzni, Institut Curie, Research Center, Paris, France

ES Cells and Development
Chairperson: W. Reik, The Babraham Institute, Cambridge, United Kingdom

Heterochromatin, Centromeres, and Nuclear Position
Chairperson: T. de Lange, The Rockefeller University, New York

Germ Cells and Early Development
Chairperson: D. Page, HHMI/Whitehead Institute/MIT, Cambridge, Massachusetts

Metabolism Signaling and Homeostasis
Chairperson: D. Littman, HHMI/New York University School of Medicine, New York

Evolution
Chairperson: S. Lindquist, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

RNA-Based Immunity
Chairperson: J. Doudna, HHMI/University of California, Berkeley

Dorcas Cummings Lecture: The Genetic Legacy of Neanderthals
S. Pääbo, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany

Genome Stability
Chairperson: A. Ferguson-Smith, University of Cambridge, United Kingdom
Target Location and Genome Engineering
Chairperson: N. Proudfoot, University of Oxford, United Kingdom

Summary
S. Gasser, Friedrich Miescher Institute for Biomedical Research, Switzerland

C. Glass, K. Adelman
MEETINGS

Systems Biology: Global Regulation of Gene Expression

January 28–February 1

231 Participants

ARRANGED BY

Timothy Hughes, University of Toronto, Canada
Christina Leslie, Memorial Sloan Kettering Cancer Center, New York
John Stamatoyannopoulos, University of Washington, Seattle
Sarah Teichmann, European Bioinformatics Institute/EMBL United Kingdom, Cambridge

Systems biology aims to understand the emergent properties of molecular networks in cells by using systematic and global approaches. One of the most actively researched areas of systems biology in recent years has been global regulation of gene expression, which coordinates complex metabolic and developmental programs in organisms. This ninth conference, like those in previous years, captured the continuing rapid progress and many new discoveries in this young and exciting field. A total of 229 scientists attended the 4-day meeting, which featured 48 talks and 128 poster presentations covering a broad range of topics. The speakers, poster presenters, and other conference attendees comprised a mix of students, postdocs, and PIs at all levels. Two keynote speeches were exciting highlights of the meeting. The first was presented by Dr. Naama Barkai, who has been a leader in the area of systems biology of gene expression for many years. Dr. Barkai described the first quantitative dissection of the contributions of initiation and elongation of transcription versus translation to yeast fitness under various growth conditions (phosphate starvation, nitrogen starvation, etc.). Dr. Orkin presented his work on understanding hematopoiesis and unraveling the correct lineage tree, as well as a careful dissection of the switch in expression from fetal to adult globin chains, with relevance to sickle cell anemia.

This year’s meeting featured many new techniques using next-generation sequencing—arguably the largest advance being in single-cell genomics technologies. This exciting new area takes the regulation of gene expression from averages of hundreds or millions of cells right down to the resolution of a single cell. Many of the presentations also described new approaches to the analysis of data, and these computational advances are driving the field forward at a tremendous pace. An example of this is the new ChIP-Seq normalization techniques that allow diverse data sets to be compared collectively, presented by the Ideker lab. Further advances relative to previous years are the ubiquitous inclusion of genome-wide association studies of disease-related mutations, so that many studies of gene expression are now directly linked to human phenotypes.

The integration of genome biology with bioinformatics, genetics, and single-cell approaches at this conference is testament to this very exciting era in research on gene expression regulation.

This meeting was funded in part by the National Human Genome Research Institute.
PROGRAM

Regulatory Elements and Their Regulation
Chairperson: S. Mahony, Pennsylvania State University, University Park

Epigenetics and Chromatin
Chairperson: T. Furey, University of North Carolina, Chapel Hill

Cellular Decision-Making
Chairperson: M. Hemberg, Wellcome Trust Sanger Institute, Hinxton, United Kingdom

Keynote Address
J. Lee, Massachusetts General Hospital, Boston

Evolution and Variation
Chairperson: S. Roy, University of Wisconsin, Madison

Transcription Factors
Chairperson: I. Ladunga, University of Nebraska, Lincoln

RNA and Its Regulation
Chairperson: R. Amit, Technion-Israel Institute of Technology, Haifa

Keynote Address
K. Yamamoto, University of California, San Francisco

Networks and Global Analyses
Chairperson: S. Aerts, University of Leuven, Belgium

The Rio Mar Ballroom
This meeting marked the departure of co-chair Galit Lahav, who was replaced by Jennifer Lippincott-Schwartz. The general format of previous meetings was maintained this year, with a continued emphasis on quantitative approaches to cell biology, combining mathematical and computational modeling with quantitative measurements and analysis. A set of themed sessions combined talks from invited speakers with shorter contributed talks. In addition to the scientific talks, there was a day-long premeeting tutorial session introducing computational approaches and software for modeling cellular and subcellular dynamics. Additional tutorials were interspersed with themed sessions throughout the regular meeting. These tutorials were well-attended and received, and it was agreed by the organizers that these should be a regular feature of meetings moving forward and that one of the regular themed sessions in future meetings should be devoted to talks highlighting the use of these approaches to tackle specific biological problems. Thus, the 2017 meeting will be co-chaired by James Faeder, a leader in the computational modeling community.

On Tuesday night, Cliff Brangwynne and Amy Gladfelter gave back-to-back talks from physical and biological perspectives highlighting emerging roles for phase separation of RNA/protein droplets in organizing cell polarity, growth, and division in a variety of cellular contexts.

On Wednesday morning, Alex Mogilner discussed recent work synthesizing models and experiments to understand symmetry breaking in motile cells through the interplay of contractility and cell-substrate adhesion. Johanns Bellaiche and Jen Zallen then highlighted their recent efforts to understand how patterned gene expression across epithelial tissues is used to sculpt regional
differences in cellular force generation to produce robust tissue morphogenesis. Following a very lively afternoon poster session, Marc Kirschner gave a keynote address highlighting exciting new work that combines single-molecule imaging with quantitative modeling to unravel the mechanistic basis for specificity and efficiency in the ubiquitylation pathway. Marc set a wonderful tone throughout the meeting with a continuous stream of insightful questions and comments. Extending the theme of Marc’s talk, Julie Biteen described the very elegant use of in vivo single-molecule imaging to probe the microscale dynamics of transcriptional regulation in bacterial cells.

Vito Quaranta chaired an exciting session Thursday morning on the use of models to assess efficacy of cancer therapies. This was followed by a highly complementary session on cell population dynamics headlined by Wenying Shou, who described elegant work using engineered yeast communities to understand the dynamic interplay between cheaters and cooperators.

Sean Sun led an exciting afternoon session on quantitative analysis of cell shape change and motility by summarizing novel insights into the role of osmotic force in these processes coming from modeling and experiments.

On Friday morning, we returned to cytoskeletal dynamics. Dan Needleman described the elegant use of quantitative microscopy and physical theory to understand self-organization of the mitotic spindle, and Tom Shemesh described the cytoskeletal origins of chiral deformations in motile cells. In the second and final session, we heard a series of beautiful talks, headed by K.C. Huang and Michael Kozlov, highlighting how the shapes of cells and internal organelles emerge from the interplay of local biochemistry and physical constraints.

Overall, the meeting was a resounding success. The quality of contributed talks was amazingly high and represented a broad diversity of questions and approaches. The organizers are looking forward to 2017!

This meeting was funded in part by the National Science Foundation.
Cancer Biology
Chairperson: V. Quaranta, Vanderbilt University School of Medicine, Nashville, Tennessee

Cell Population Dynamics
Chairperson: W. Shou, Fred Hutchinson Cancer Research Center, Seattle, Washington

Cell Mechanics and Motility
Chairperson: S. Sun, Johns Hopkins University, Baltimore, Maryland

Cytoskeletal Dynamics II
Chairpersons: A. Bershadsky, Weizmann Institute of Science, Rehovot, Israel; D. Needleman, Harvard University, Cambridge, Massachusetts

Cell Organization and Growth
Chairpersons: K.C. Huang, Stanford University, California; M. Kozlov, Tel Aviv University, Israel
Genomic information in cells and organisms is translated into phenotypes by complex and highly dynamic molecular networks formed by proteins, nucleic acids, and small molecules. A systems-level understanding of biological systems or disease phenotypes, as well as the design of rational biotechnological or pharmaceutical interventions in humans, crops, and microbes, hinges on our knowledge of these networks.

Biological network science aims to understand these molecular networks at the interface between genotype and phenotype. As molecular networks still remain largely unknown, a prerequisite for their functional investigation and an important goal of network science is to experimentally map or computationally infer the wiring of cells. A second major area is the mechanistic characterization of smaller network modules, translating large-scale network connectivity into molecular mechanisms and forming a basis for the development of quantitative and predictive models. Finally, network science also aims to develop and apply statistical tools to extract insights from known biological networks to identify disease-causing genes and modules, identify targets for intervention, and decipher the fundamental principles that underlie biological systems and their evolution.

Several novel elements were introduced at the 2015 meeting, including two open discussions aimed at community reflection and discussion on future trends in network science and on innovative publishing models. In addition, an informal “Lunch with a PI” event was introduced to foster contact between established PIs and young scientists. More than 30 graduate students and young postdocs had the opportunity to talk in small groups with around 10 established scientists (including one from the pharmaceutical industry and one from scientific editing) about topics ranging from career advice to specific scientific discussions. All these elements were very much welcomed by the attendees and resulted in active discussions that were not limited to the time frame of the respective event but continued throughout the remainder of the meeting.

The program opened on Tuesday with a keynote address from Brenda Andrews of the University of Toronto. Dr. Andrews’ inspiring presentation illustrated how layering different types of systematic data collection can lead to all sorts of new insights about how cells function. In the following days, the program continued with 17 invited presentations and 26 short talks selected from submitted abstracts, all of them of outstanding quality and many given by young scientists at the postdoctoral and Ph.D. student level. The presentations covered many aspects of network and systems biology. Some described efforts toward generating and analyzing systematic data sets of many types of biological interactions, both in human cells and in various model organisms. Other presentations focused on sophisticated computational analyses of smaller dynamical networks. Insights were many and diverse, from how to leverage our knowledge of micro-organismal metabolic networks to optimize treatment strategies for infections, to experiments that reveal a physical map...
of a human cell; from determining the function of yet uncharacterized genes to determining organizing principles of gene regulatory networks; from ways to quantify the central dogma of biology to new ways to leverage patient data to improve diagnosis and cancer prognosis. As in our previous meetings, these presentations highlighted how much progress has been made in recent years and, at the same time, how open questions, exciting opportunities, and technological challenges remain.

Finally, late Saturday morning, we had the pleasure to hear the second keynote address from Mike Tyers of the University of Montreal. Dr. Tyers gave a beautiful presentation, first anchoring the science of network biology with early ideas from Conrad Waddington published in the 1950s, then presenting thought-provoking work from his lab on using network science to identify potential drug synergies, and finally putting forward some of the challenges that lie ahead as the science moves from descriptive networks to predictive networks.

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 Address
M. Vidal, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

Gene Regulatory Networks
Chairperson: C. Myers, University of Minnesota, Minneapolis
Protein Interaction Networks I
Chairperson: P. Aloy, Institute for Research in Biomedicine, Barcelona, Spain

Metabolic Networks
Chairperson: P. Braun, Technical University of Munich, Germany

Protein Interaction Networks II
Chairperson: A.-C. Gavin, European Molecular Biology Laboratory, Heidelberg, Germany

Genetic Interaction Networks
Chairperson: T. Ideker, University of California, San Diego

Networks and Disease I
Chairperson: M. Walhout, University of Massachusetts Medical School, Worcester

Signaling Networks
Chairperson: S. Gaudet, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts

Networks and Disease II
Chairperson: B. Andrews, University of Toronto, Canada

Networks and Evolution
Chairperson: M. Springer, Harvard Medical School, Boston, Massachusetts

Panel Discussion: What Is the Future of Publications for Systems Network Data Sets and Models?

Networks and Disease III
Chairperson: B. Lehner, Center for Genomic Regulation, Barcelona, Spain

Keynote Address
M. Tyers, Université de Montréal, Montréal, Canada
This was the second conference hosted at Cold Spring Harbor, following one in 2013 and two previous meetings in this series held in Ireland in 2009 and 2011. The main goal of this meeting was 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.

We had 20 invited talks from world leaders in diverse fields and an additional 18 talks selected from submitted abstracts. These talks highlighted cutting-edge research in specific fields, and it was also notable how many of them were integrated 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 Ann Graybiel, described her long-running work on the structure and function of the circuits of the striatum. Linking cutting-edge neuroanatomy and physiology with incisive behavioral experiments, informed by cognitive science, this talk illustrated the deep level of understanding that can be achieved in reverse engineering neural circuits. The second talk was by Nobel laureate Thomas Südhof, who presented a tour de force overview of his work on the molecular mechanisms of synapse specification, and a detailed look at more recent work, highlighting unexpected roles for synaptogenic molecules. Together, these two talks illustrated the necessity and the possibility for linking analyses across multiple levels, from molecular and cellular to circuits, systems, and behavior.

There were 86 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.

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

Development: Early Events
Chairperson: J. Huang, Cold Spring Harbor Laboratory

Synapse Formation and Plasticity
K. Mitchell, Trinity College Dublin, Ireland

Human Brain Development
Chairperson: F. Polleux, Columbia University, New York

Keynote Address
A.M. Graybiel, Massachusetts Institute of Technology, Cambridge

Neurodevelopmental Disorders
Chairperson: P. Arlotta, Harvard University, Cambridge, Massachusetts

Neural Dynamics
Chairperson: J. Gordon, Columbia University, New York

Circuits: Function and Dysfunction
Chairperson: R. Cossart, INSERM, Marseille, France

Systems: Function and Dysfunction
Chairperson: G. Buzsaki, New York University Neuroscience Institute, New York

Keynote Address
T.C. Südhof, Stanford University School of Medicine, California
RNA and Oligonucleotide Therapeutics

April 8–11 92 Participants

ARRANGED BY
Annemieke Aartsma-Rus, Leiden University Medical Center, The Netherlands
Arthur Krieg, Checkmate Pharmaceuticals, Cambridge, Massachusetts
Laura Sepp-Lorenzino, Alnylam Pharmaceuticals, Cambridge, Massachusetts
Bruce Sullenger, Duke University, Durham, North Carolina

This first conference on RNA and Oligonucleotide Therapeutics 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 siRNAs and microRNAs as potential drugs and targets. The all-important issue of RNA therapeutics delivery by a range 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 in 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 by Nobel laureate Dr. Sydney Altman and 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 AUM LifeTech, Inc.
PROGRAM

Keynote Address
C.A. Gersbach, Duke University, Durham, North Carolina

Single-Stranded Oligonucleotides
Chairpersons: A. Aartsma-Rus, Leiden University Medical Center, The Netherlands; A. Krainer, Cold Spring Harbor Laboratory

Aptamer and Chemistry
Chairpersons: B. Sullenger, Duke University, Durham, North Carolina; S. Wilcox, SomaLogic, Inc., Boulder, Colorado

mRNA and Exon Skipping
Chairpersons: M. Moore, Novartis Vaccines, Cambridge, Massachusetts; A. Geall, HHMI/University of Massachusetts Medical School, Worcester

dsRNA
Chairpersons: L. Sepp-Lorenzino, Alnylam Pharmaceuticals, Cambridge, Massachusetts; B. Brown, Dicerna, Cambridge, Massachusetts

ncRNA
Chairperson: D. Corey, University of Texas Southwestern Medical Center, Dallas

Genome Editing and Novel Mechanisms
Chairpersons: C. Gersbach, Duke University, Durham, North Carolina; A. Krieg, Checkmate Pharmaceuticals, Cambridge, Massachusetts
This inaugural meeting held during warm and sunny spring days after a harsh Long Island winter was developed to fill a clear need for enhanced interaction between basic researchers studying fundamental questions in the field of immunology and translational/clinical researchers who have taken advantage of this fundamental knowledge for the development of innovative new therapies for the treatment of cancer, autoimmune diseases, and infectious diseases. The meeting included 235 participants, eight sessions with oral presentations by invited speakers and speakers selected from submitted abstracts, and two lively poster sessions. Updates by James Allison (MD Anderson Cancer Center), Padmanee Sharma (MD Anderson Cancer Center), and others on the remarkable success of checkpoint blockade immunotherapies for the treatment of melanoma and other devastating cancers represented a highlight of the conference. Although complete long-lasting remission following treatment with single checkpoint blockade antibodies had been reported in 20%–30% of melanoma patients over the past few years, preliminary data from combination checkpoint therapy trials raise the possibility of long-term survival of >50% of patients and possibly as high as 80%. Fundamental studies of tumor neoantigens and the tumor microenvironment were also discussed and have the potential to lead to a broader range of immunotherapeutic options for cancer patients. With respect to infectious diseases, Michel Nussenzweig (Rockefeller University) presented encouraging preliminary data on a novel antibody-based immunotherapy approach for the treatment of HIV infection.
Other studies of infectious diseases focused on immune exhaustion, T regulatory cells, and T-cell fate decisions and plasticity, all of which have the potential to lead to novel treatments—not only for bacterial and viral infection, but also for cancer. Notably, the checkpoint blockade therapies for cancer emerged from fundamental studies of immune responses to microbial infection, demonstrating the importance of interactions between immunologists studying different diseases and topics. Autoimmune and chronic inflammatory diseases represented another major emphasis of the meeting. One entire session was devoted to a series of exciting presentations describing the interplay between the immune system and microorganisms that naturally reside in the gut; an active area of investigation is to understand how the human immune system suppresses its response to the gut microbiota in healthy individuals, and how dysregulation of the microbiota or the immune system lead to inflammatory diseases. Cold Spring Harbor Laboratory proved to be an ideal venue for promoting interactions between leading fundamental and translational immunologists, not only because of the targeted selection of topics for inclusion in the program, but also because of the intimate setting, in which all participants dine together at Blackford Hall and meet for extended discussions after the evening sessions in the only accessible bar.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.
PROGRAM

Host–Pathogen Interactions
Chairperson: F. Sallusto, Institute for Research in Biomedicine, Bellinzona, Spain

Immunity and Immune Pathology
Chairperson: G. Griffiths, Cambridge Institute for Medical Research, United Kingdom

NIH Funding for Young Investigators
Chairperson: C. Malia, NIAID, National Institutes of Health, Bethesda, Maryland

Neo-Antigens and the Tumor Microenvironment
Chairperson: M. Karin, University of California, San Diego

Intestinal Immune Responses and Homeostasis
Chairperson: F. Powrie, University of Oxford, United Kingdom

Adoptive Cell Therapy
Chairperson: M. Sadelain, Memorial Sloan Kettering Cancer Center, New York

Checkpoint Blockade Therapies
Chairperson: J. Allison, MD Anderson Cancer Center, Houston, Texas

Autoimmunity and T Regulatory Cells
Chairperson: A. Rudensky, Ludwig Center at Memorial Sloan Kettering Cancer Center, New York

Antimicrobial Immunity and Vaccines
Chairperson: M. Nussenzweig, HHMI/Rockefeller University, New York
The Ubiquitin Family

April 21–25        198 Participants

ARRANGED BY        Ronald Hay, University of Dundee, United Kingdom
                   Ron Kopito, Stanford University, California
                   Cynthia Wolberger, Johns Hopkins University School of Medicine, Baltimore, Maryland

Following the successful inauguration of this series in 2003, this sixth meeting 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 included 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 neurodegenerative diseases and 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 was capped by two exciting keynote lectures from Brenda Schulman and Ivan Dikic, who have contributed seminal results to our understanding of the structural biological and cell physiological basis of the ubiquitin system. The meeting attracted 198 scientists who engaged in lively discussions concerning the roles of ubiquitin in protein turnover and 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, as well as newly discovered roles for phosphorylation of ubiquitin. There is also a growing understanding of non-proteolytic 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, 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 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. Major new structures of nucleosomes bound to ubiquitin conjugating and deconjugating enzyme complexes provided the first glimpses of chromatin-targeted ubiquitin signaling mechanisms. 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, including of 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 that small molecules can target the ubiquitin-proteosome, at virtually every level of the pathway. In summary, this meeting continues 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.

This meeting was funded in part by Boston Biochem.
PROGRAM

Control of Cellular Functions by Ubiquitin and UBLs
Chairpersons: D. Barford, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom; D. Komander, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom

Keynote Address
B. Schulman, St. Jude Children’s Research Hospital, Memphis, Tennessee

Quality Control
Chairpersons: A. Bertolotti, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom; W. Harper, Harvard Medical School, Boston, Massachusetts

Control of E3 Ligase Activity
Chairpersons: C. Lima, Memorial Sloan Kettering Cancer Institute, New York; N. Thoma, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland

Regulation by Ubiquitin and SUMO
Chairpersons: M. Rape, HHMI/University of California, Berkeley; M. Gyrd-Hansen, University of Oxford, United Kingdom

Mechanism of Action of E3 Ligases
Chairpersons: R. Klevit, University of Washington, Seattle; K. Rittinger, MRC National Institute for Medical Research, London, United Kingdom

Removal of Ubiquitin and UBLs
Chairpersons: T. Sixma, Netherlands Cancer Institute, Amsterdam; M. Nakanishi, Nagoya City University, Japan

Autophagy
Chairpersons: R. Youle, National Institutes of Health, Bethesda, Maryland; F. Melchior, Zentrum für Molekulare Biologie Heidelberg, Germany

Keynote Address
I. Dikic, Goethe University Medical School, Germany

Proteasome
Chairpersons: A. Martin, University of California, Berkeley; K.H. Darwin, New York University School of Medicine, New York
Telomeres and Telomerase

April 28–May 2 319 Participants

ARRANGED BY
Titia de Lange, The Rockefeller University, New York
Julia Cooper, National Cancer Institute, Bethesda, Maryland
Roger Reddel, Children's Medical Research Institute, Westmead, Australia

The conference consisted of eight sessions of talks and two poster sessions. As in 1999, 2001, 2003, 2005, 2007, 2009, 2011, and 2013, 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-minute presentation themselves or having a member of their labs 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 given by graduate students and postdoctoral fellows. Attendance exceeded 300 participants, a large proportion of whom presented the 143 posters and 76 talks.

The talks and posters covered many aspects of telomere and telomerase biology, including telomere replication, telomerase structure, enzymology, recruitment and regulation, telomere length homeostasis, repair of dysfunctional telomeres, role of telomeres in cancer and aging, diseases associated with short telomeres, telomerase-independent telomere maintenance, telomeric RNA, and chromatin at chromosome ends.

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 2017.

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

Telomerase, Telomeres, and Cancer
Chairpersons: S. Gasser, Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland; C. Greider, Johns Hopkins University, Baltimore, Maryland

Repair of Dysfunctional Telomeres
Chairpersons: E. Lazzerini Denchi, The Scripps Research Institute, La Jolla, California; V. Lundblad, Salk Institute for Biological Studies, La Jolla, California
Recombination at Telomeres and the Alt Pathway I
Chairpersons: T. Bryan, Children’s Medical Research Institute, Westmead, Australia; J. Shay, University of Texas Southwestern Medical Center, Dallas

Recombination at Telomeres and the Alt Pathway II
Chairpersons: V. Zakian, Princeton University, New Jersey; L. Rudolph, Leibniz Institute for Age Research, Jena, Germany

Chromatin at Chromosome Ends
Chairpersons: V. Zakian, Princeton University, New Jersey; L. Rudolph, Leibniz Institute for Age Research, Jena, Germany

Telomere Replication
Chairpersons: J. Lingner, EPFL, Lausanne, Switzerland; P. Baumann, HHMI/Stowers Institute for Medical Research, Kansas City, Missouri

Telomeres, Telomerase RNA, and Human Disease
Chairpersons: S. Boulton, London Research Institute, South Mimms, United Kingdom; A. Bertuch, Baylor College of Medicine, Houston, Texas

Telomere Length Homeostasis and Telomerase Recruitment I
Chairpersons: S. Boulton, London Research Institute, South Mimms, United Kingdom; A. Bertuch, Baylor College of Medicine, Houston, Texas

Telomere Length Homeostasis and Telomerase Recruitment II
Chairpersons: D. Shore, University of Geneva, Switzerland; M.T. Teixeira, CNRS, Paris, France

The Structure of Telomeres and Telomerase
Chairpersons: T. Cech, HHMI/University of Colorado, Boulder; D. Rhodes, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
This meeting brought together leaders and younger scientists in the field of sociogenomics—that is, applying genomics of social insects to study a broad range of topics from social behavior, to microbiomes, to pollinator health, to evolution and phenotypic plasticity.

The three keynote speakers represented three eusocial insect lineages: ants, bees, and termites. They presented different approaches for using genomes and genomic tools to understand the genomic architecture of behavior and social organization. All three emphasized the need to use a systems approach, coupled with in-depth study of individual genes or networks to understand the genetic architecture of complex phenotypes (e.g., group-level traits such as division of labor or social organization).

The Data Blitz gave 18 younger researchers (mostly Ph.D. students and postdocs) the opportunity to present key findings and draw attention to some of the excellent work presented in the poster session.

The diverse species represented clearly showed that sociogenomics has exploded within the last 10 years. We went from one genome (*Apis mellifera*) to more than 50 sequenced social insect genomes and many more in the pipeline. However, discussion emphasized that the field is in desperate need of better gene annotation methods that do not solely rely on genome annotations of a few model organisms like *Drosophila*. With so many social insect genomes, we can now start to look for commonalities and differences in the evolution of independently evolved eusocial insect lineages.

Social insects are models for studying phenotypic plasticity and epigenetic regulation. However, convergently evolved eusocial insect lineages use different mechanisms of epigenetic regulation.
For example, honey bees apparently use DNA methylation extensively, whereas Polistes wasps are missing key enzymes (DNMT) needed for DNA methylation and show almost no DNA methylation. Ants seem to be in-between, but there is evidence that histone modifications play a role in epigenetic regulation of subcastes. The validity of several DNA methylation studies was hotly discussed, and several participants requested better quality-control measures and documentation in future studies in this research area of sociogenomics.

Some eusocial insects (e.g., leafcutter ants and termites) have tightly evolved relationships with microbes and are at the forefront of microbiome studies. New studies emphasized that more eusocial insects have significant interactions with microbes than previously known, and these interactions could significantly impact social organization. More research and attention needs to be paid to these interactions. For example, genomics has significantly advanced the study of honeybee pathosystems and is providing new insights into disease, immunity, and pollinator health, in general.

Major issues for the future are data archiving and accessibility. NCBI has been the main repository, but it is not clear whether this will be sustainable given the hundreds or even thousands of –omics data sets being generated in the coming decade. There was discussion but no consensus on future directions: Should we support existing resources such as Hymenopteran Base or Flybase, or should we switch to a more self-organized and open-source model? This is a general problem in the fast developing “genomics era” that is not limited to social insect research.

PROGRAM

Keynote Address
G.E. Robinson, University of Illinois, Urbana

Novel Techniques and Bioinformatic Advances and Applications to Social Insect Biology
Chairpersons: C. Grozinger, Pennsylvania State University, University Park; G. Zhang, University of Copenhagen, Denmark

Keynote Address
J. Korb, University of Freiburg, Germany

Comparative Genomics of Insect Sociality
Chairpersons: K. Kapheim, Utah State University, Logan; A. Toth, Iowa State University, Ames

Physiological and Developmental Basis of Social Insect Phenotypes I
Chairpersons: K. Hartfelder, Universidade de Sao Paulo, Brazil; E. Abouheif, McGill University, Montreal, Canada
Physiological and Developmental Basis of Social Insect Phenotypes II
Chairpersons: K. Hartfelder, Universidade de Sao Paulo, Brazil; E. Abouheif, McGill University, Montreal, Canada

Molecular Evolution of Insect Societies I
Chairpersons: A. Zayed, York University, Toronto, Canada; J. Gadau, Arizona State University, Tempe

Keynote Address
L. Keller, University of Lausanne, Switzerland

Molecular Evolution of Insect Societies II
Chairpersons: A. Zayed, York University, Toronto, Canada; J. Gadau, Arizona State University, Tempe

Data Blitz
Chairpersons: J. Gadau, Arizona State University, Tempe; G. Bloch, The Hebrew University of Jerusalem, Israel; A. Toth, Iowa State University, Ames

Neurobiology and Behavior of Social Insects
Chairpersons: R. Scheiner, University of Wurzburg, Germany; G. Bloch, The Hebrew University of Jerusalem, Israel

Social Insect Microbiomes and Immunity
Chairperson: J. Evans, USDA-ARS, Beltsville, Maryland

Epigenetics, Gene Regulation, and Social Organization
Chairperson: M. Goodisman, Georgia Institute of Technology, Atlanta
The Biology of Genomes

May 5–9 558 Participants
ARRANGED BY Ewan Birney, EBI/EMBL, United Kingdom
Michel Georges, University of Liege, Belgium
Elaine Mardis, Washington University School of Medicine, St. Louis, Missouri
Molly Przeworski, Columbia University, New York

This annual meeting marked the 28th annual gathering of genome scientists at the Laboratory. Researchers from around the world attended the meeting, with more than 385 abstracts presented describing a broad array of topics relating to the functional analysis, comparative characterization, and interpretation of genomes from diverse organisms. The meeting featured talks about gene regulation across mammalian species, the detection of novel open reading frames (ORFs) from ribosome footprinting data, and three-dimensional mapping of the human genome to understand chromatin looping and other aspects of functional genomics. Cancer genomics featured prominent studies of single-cell genomics, liquid biopsy technology, and clonal evolution. The translational genomics and genetics session highlighted whole-genome sequencing studies of cattle, maize studies of open chromatin, and the human pancreatic islet transcriptome, among others. The majority of computational genomics efforts focused on single-cell data analysis in mammalian development, nucleotide-level resolution of transcription, and methods to explore genetic variation in millions of human genomes. Sessions on population and evolution/nonhuman genomics introduced new data on African–American population structure, patterns of mosaicism in development, links between immune response regulation and adaptation to pathogen pressure, imprinting in the opossum, high-altitude adaptations in Tibetans, and the evolution of rattlesnake venom. These topics underscore the broad-scale impact of massively parallel sequencing technology and techniques upon our burgeoning comprehension of biological questions.

All sessions were well attended, stretching the capacity of the CSHL facilities. George Davey Smith and Francis S. Collins gave the keynote presentations.

The ELSI (Ethical, Legal, and Social Implications) panel was moderated by Lawrence Brody and the area of discussion was “Genomic Data Sharing: Past, Present, and Future.” The panelists included Laura Rodriguez, Eric Campbell, and Daniel O’Connor.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health; Illumina; and Swift Biosciences.
PROGRAM

Functional Genomics
Chairpersons: R. Darnell, New York Genome Center, New York; G. Petukhova, Uniformed Services University of the Health Sciences, Bethesda, Maryland

Cancer and Medical Genomics
Chairpersons: C. Dive, University of Manchester, United Kingdom; E. Mardis, Washington University School of Medicine, St. Louis, Missouri

Genetics of Complex Traits
Chairpersons: J. Flint, University of Oxford-Wellcome Trust Centre for Human Genetics, United Kingdom; N. Soranzo, Wellcome Trust Sanger Institute, Cambridge, United Kingdom

Computational Genomics
Chairpersons: J. Marioni, EBI-EMBL, Hinxton, Cambridge, United Kingdom; F. Michor, Harvard School of Public Health/Dana Farber Cancer Institute, Boston, Massachusetts

ELSI Panel and Discussion: Genomic Data Sharing: Past, Present, and Future
Moderator: L. Brody, NHGRI, National Institutes of Health, Bethesda, Maryland

Population Genomics
Chairpersons: K. Bomblies, Harvard University, Cambridge, Massachusetts; A. Price, Harvard T.H. Chan School of Public Health, Boston, Massachusetts

Evolutionary and Non-Human Genomics
Chairpersons: D. Bachrorg, University of California, Berkeley; J. Willis, Duke University, Durham, North Carolina

Guest Speakers
G. Davey Smith, University of Bristol, United Kingdom; F.S. Collins, National Institutes of Health, Bethesda, Maryland

Translational Genomics and Genetics
Chairpersons: B. Hayes, Department of Environment and Primary Industries, Melbourne, Australia; D. Lo, Chinese University of Hong Kong
Cancer is now recognized as an abnormal organ where multiple signals from multiple cell types cooperate during tumor initiation and metastatic progression. Significant progress is being made on identifying and characterizing all cell types composing a tumor, understanding the mechanisms by which these cells interact with each other, developing ways to image the changes in a tumor, and exploring therapeutic opportunities that take into account the non-cancer cells. Thus, there is an emergence of a need for an integrative approach to 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; biologists who are developing mouse and organoid models to study human cancers; and medical oncologists who are using all this information to best treat patients. Our keynote speaker, Mina Bissell, argued that phenotype trumps genotype in cancer progression. Many additional speakers were prominent pioneers and thought leaders in their fields and collectively led stimulating discussions during the meeting. In addition, we held a lively panel discussion entitled “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, logistical, fiscal, and ethical limitations. Finally, we introduced a career mentoring session for trainees, and this appeared to be very popular. The meeting, as a whole, was received very well and the participants enjoyed both the diversity and the depth of topics covered.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.

PROGRAM

Keynote Address
M.J. Bissell, Lawrence Berkeley National Laboratory, Berkeley, California

Tumor Heterogeneity: Genetic and Epigenetic
Chairpersons: J. Bradner, Dana-Farber Cancer Institute, Boston, Massachusetts; E. Mardis, Washington University School of Medicine, St. Louis, Missouri

Translational Trial/Personalized Cancer Therapy
Chairpersons: L. Diaz, Johns Hopkins University School of Medicine, Baltimore, Maryland; D. Solit, Memorial Sloan Kettering Cancer Center, New York

Panel Discussion: Will the Ineffectiveness of Oncology Trials be Rescued by Personalized Medicine?
Diagnostic Imaging and Drug Delivery
Chairpersons: S. Lowe, Memorial Sloan Kettering Cancer Center, New York; D. Piwnica-Worms, University of Texas MD Anderson Cancer Center, Houston

Cancer Immunology
Chairpersons: T. Chan, Memorial Sloan Kettering Cancer Center, New York; G. Freeman, Dana-Farber Cancer Institute, Boston, Massachusetts

Cancer as a Systemic Disease
Chairpersons: M. Egeblad, Cold Spring Harbor Laboratory; D. Lyden, Weill Cornell Medical College, New York

Tumor Microenvironment and Metastasis
Chairpersons: J. Joyce, Memorial Sloan Kettering Cancer Center, New York; A. Welm, Oklahoma Medical Research Foundation, Oklahoma City

Metabolism
Chairperson: T. Mak, University of Toronto, Canada

Signaling Networks and Therapeutics
Chairpersons: H. Clevers, Hubrecht Laboratory and University Medical Center, Utrecht, The Netherlands; J. Engelman, Massachusetts General Hospital, Boston
Both of the above organizers are regular attendees who, like many of the participants, have “grown up” at this meeting and within its distinct community of scientists. Indeed, a characteristic feature of the Retroviruses meeting remains the high number of repeat attendees who come each year, where friendships and collaborations are both initiated and maintained.

As keynote speakers, the organizers chose to recognize two of the most prominent and highly respected retrovirologists, Drs. Malcolm Martin and Susan Ross Malcolm. They presented an overview of his many contributions to basic retrovirology and HIV/AIDS research with a sense of humor and fascinating personal anecdotes. Malcolm’s talk was very well-received by the attendees, many of whom found it inspirational. Susan also presented a very well-received and humorous talk that highlighted her work on mouse mammary tumor virus (MMTV) and innate immunity. Her talk continued a recent trend toward emphasizing the host–pathogen relationship during retroviral infections.

Three annual prizes, originally instituted and endowed by the community of regular attendees with assistance from CSH Meetings staff, were also awarded. These prizes memorialize three of our former colleagues who passed away unexpectedly in recent years (the Daniel Wolf Prize, the Ute Von Schwedler Prize, and the Andy Kaplan Prize), and serve to encourage up-and-coming scientists at the graduate and postdoc/junior levels. The prizes are also a testament to the community nature of the CSH Retroviruses meeting and the continuity of the attendees over the years. The award recipients this year were Sebla Kutluay (Andy Kaplan Prize), Kellie A. Jurado (Uta von Schwedler Prize), and Shilpa Iyer (Daniel Wolf Prize).
As previously, the meeting was organized into 13 sessions (10 oral and three posters); the presentations featured unpublished, primarily basic research. The sessions focused on traditional areas, such as specific aspects of the retrovirus life cycle, as well as on classes of host defenses, especially specific restriction factors and the viral proteins that counteract them. A general session on Entry/Env opened the meeting, and a general session on Innate Immunity and Pathogenesis closed the meeting. The organizers attempted to distribute talks that could fit in alternative sessions in a manner that led to each session having “something for everyone.”

Scientific highlights of the meeting included the identification of the SERINC3/5 proteins as the cellular factors that account for infectivity enhancement by the HIV-1 Nef and MLV GlycoGag proteins and novel insights into the mechanisms of innate sensing of retroviral infection in cells. A range of basic information regarding the retroviral life cycle, such as the role of matrix in envelope glycoprotein incorporation and virus assembly, was also presented. As previously, the meeting also exemplified new technology that could be applied both to the discipline of retrovirology and to other areas of research.

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, Inc.

PROGRAM

Entry/Env
Chairpersons: T. Hatzioannou, Aaron Diamond AIDS Research Center, New York; M. Johnson, University of Missouri, Columbia

Postentry/Capsid
Chairpersons: E. Campbell, Loyola University, Chicago, Illinois; M. Yamashita, Aaron Diamond AIDS Research Center, New York

Vpx / SAMHD1
Chairpersons: K. Bishop, Francis Crick Institute at NIMR, London, United Kingdom; L. Wu, The Ohio State University, Columbus

Keynote Address
M. Martin, NIAID, National Institutes of Health, Bethesda, Maryland

APOBEC and Beyond
Chairpersons: R. Harris, University of Minnesota, Minneapolis; V. Simon, Icahn School of Medicine, New York

Reverse Transcription and Integration
Chairpersons: M. Roth, Rutgers-Robert Wood Johnson Medical School, Piscataway, New Jersey; K. Yoder, The Ohio State University, Columbus

Fourth Annual Uta von Schwedler Prize for Retrovirology
Presented by: W. Mothes, Yale University, New Haven, Connecticut
Awarded to: K.A. Jurado, Dana-Farber Cancer Institute, Boston, Massachusetts
Tetherin, IFITM, and Nef
Chairpersons: S.-L. Liu, University of Missouri, Columbia; M. Pizzato, University of Trento, Italy

RNA Packaging, Assembly
Chairpersons: J. Lingappa, University of Washington, Seattle; J. Mak, Deakin University, Geelong, Australia

Keynote Address
S.R. Ross, University of Pennsylvania, Philadelphia

RNA/Host Cofactors
Chairpersons: A. Cochrane, University of Toronto, Canada; L. Parent, Pennsylvania State University College of Medicine, Hershey, Pennsylvania

Ninth Annual Andy Kaplan Prize
Presented by: W. Johnson, Boston University, Massachusetts
Awarded to: S. Kutluay, Washington University School of Medicine, St. Louis, Missouri

Assembly, Budding, and Maturation
Chairpersons: C. Carter, Stony Brook University, New York; N. Sherer, University of Wisconsin, Madison

Fifth Annual Daniel Wolf Prize
2014 Recipient: M. Qi, Emory University, Atlanta, Georgia
Presented by: R. Liberatore, Aaron Diamond AIDS Research Center, New York

Innate Immunity and Pathogenesis
Chairpersons: A. Finzi, CHCRUM, Université de Montréal, Canada; C. Jolly, University College London, United Kingdom
The Evolution of Sequencing Technology—A Half-Century of Progress

July 16–19
111 Participants

ARRANGED BY
Mark Adams, J. Craig Venter Institute, San Diego, California
Nigel Brown, University of Edinburgh, United Kingdom
Mila Pollock, Cold Spring Harbor Laboratory
Robert Waterston, University of Washington, Seattle

This unique meeting brought together some of the most renowned figures in molecular biology and genetics to discuss the development of DNA sequencing and its 50-year history. It was the fifth annual meeting in Cold Spring Harbor Laboratory’s History of Molecular Biology and Biotechnology series.

Sequencing refers to determining the order of the chemical base pairs in a chain of DNA. Over the years, sequencing has progressed from the slow, manual methods of the 1970s to the rapid, automated tools of the 21st century. The meeting featured a session on the late Frederick Sanger, two-time winner of the Nobel Prize in Chemistry, who is considered the father of DNA sequencing. Several of Sanger’s students, themselves eminent scientists, gave presentations on their time in Sanger’s lab and their roles in the earlier years of sequencing research. These scientists included George Brownlee, Gillian Air, and Victor Ling.

The meeting attracted 111 scientists, authors, and historians of science, students, and members of the CSHL community. A novel event, it merged science and history by framing contemporary research in terms of the field’s past, with many of the key figures in sequencing present to give insight and context. Among the attendees was Walter Gilbert, who shared one half of the 1980 Nobel Prize in Chemistry with Frederick Sanger for their development of separate methods of DNA sequencing. There were three other Nobel laureates in attendance: James Watson, who co-discovered the structure of DNA; Hamilton Smith, who co-discovered restriction enzymes; and Richard Roberts, who co-discovered split genes.

One of the meeting’s memorable moments was a panel discussion on scientists’ missteps and setbacks in developing sequencing technologies. Led by Richard Roberts, Richard McCombie, and Cheryl Heiner, the panel spurred lively debate on the nature of patenting and funding in the biological sciences. Other highlights included a presentation by Leroy Hood, who developed...
the first automated sequencing machine, on transforming Sanger’s methods into a revolutionary commercial product. Clyde Hutchison talked about his role in sequencing Phi X 174, a bacterial virus whose DNA was the first full genome to be sequenced. Miguel García-Sancho, a historian of science, talked about the history of computing technology within the sequencing field.

Although he could not attend the meeting, J. Craig Venter sent a recorded presentation in which he discussed the extraordinary pace of sequencing technology since the completion of the Human Genome Project in 2003. Shankar Balasubramanian, Jonas Korlach, and Hagan Bayley expanded on this theme by explaining recent trends and innovations in sequencing. Videos of the talks and presentations, as well as slides and posters, are available online: http://library.cshl.edu/Meetings/sequencing/.

This meeting had major support from Illumina.

**PROGRAM**

**Session I: Early Days**

J. Watson, *Cold Spring Harbor Laboratory*: Early days with DNA.

G. Brownlee, *University of Oxford, United Kingdom*: The early days of RNA sequencing at the LMB.


C. Hutchison, *J. Craig Venter Institute, San Diego, California*: Sequencing of Phi X 174.
W. Gilbert, Harvard University, Cambridge, Massachusetts: Origin of DNA sequencing.
T. Maniatis, Columbia University Medical Center, New York: The transition from RNA to DNA sequencing in the Sanger lab: The DNA sequence of the phage lambda operator/promoter regions.
J. Messing, Waksman Institute, Rutgers University, Piscataway, New Jersey

Session II: Capturing Sequences/Survey
L. Smith, University of Wisconsin, Madison: Fluorescence-based automated DNA sequencing.
N. Dovichi, University of Notre Dame, Indiana: Development of capillary electrophoresis.
M. Ronaghi, Illumina, Inc., San Diego, California: Development of pyrosequencing.
S. Balasubramanian, University of Cambridge, United Kingdom: Early development of Solexa technology: Key insights and technical breakthroughs.
J. Korlach, Pacific Biosciences, Menlo Park, California: Technical innovations of SMRT sequencing and applications of long-read sequencing.
H. Bayley, University of Oxford, United Kingdom: Nanopore sequencing.

Session III: Access to Sequence—From Past to the Future
G. Cameron, Founder and ex-Director, EMBL: DNA database prehistory.
J. Ostell, NCBI/National Center for Biotechnology Information, Bethesda, Maryland: Databases for the future.
M. Garcia-Sancho, University of Edinburgh, United Kingdom: Sequencing and computing technologies: A historical convergence.
M. Pollock, Cold Spring Harbor Laboratory: Genome legacy (preserving the history).

Session IV: Scaling to Genomes
J. Weissenbach, Genoscope—CNRG, France: Genoscope early efforts at automation.
S. Tabor, Harvard Medical School, Cambridge, Massachusetts: How enzymology enabled advances in DNA sequencing.
M. Simon, California Institute of Technology, Pasadena: Large insert cloning.
W. Elacavitch, Molecular Assemblies, Inc., San Diego, California: Technology development in scaling up Sanger sequencing.
J. Rogers, International Wheat Genome Sequencing Consortium, United Kingdom: Scaling up Sanger sequencing in the genome era.
R. Myers, HudsonAlpha Institute for Biotechnology, Huntsville, Alabama: A personal perspective on DNA sequencing from 1978 to 2015.

Y. Sakaki, University of Tokyo, Japan: From the proposal of automated DNA sequencing to the completion of the Human Genome: Japanese contribution to Human Genome sequencing.

Session V: Sequences to Genomes
J. Craig Venter, J. Craig Venter Institute, San Diego, California: Whole-genome shotgun sequencing.
H. Smith, J. Craig Venter Institute, San Diego, California: Haemophilus influenzae and the value of completeness.
P. Green, University of Washington, Seattle: Sequence quality and assembly.
J. Kent, University of California, Santa Cruz: Integrating the sequence and map into a genome.
G. Meyers, Max-Planck Institute, Germany: Shotgun assembly strategies.
S. Lewis, Lawrence Berkeley National Laboratory, California: Making sense of genomes with visualization and collaboration.

Session VI: All Roads Lead to DNA—Beyond Genomes
M. Adams, J. Craig Venter Institute, San Diego, California: Sequencing ESTs for gene discovery.
B. Wold, *California Institute of Technology, Pasadena*: Developments and applications of RNA-Seq.


P. Carninci, *RIKEN, Japan*: cDNA sequencing for genome analysis and biological interpretation.


**Panel Discussion: Steps and Mis-Steps during the Development of Sequencing Technologies**

R. McCombie, *Cold Spring Harbor Laboratory*

R. Roberts, *New England BioLabs, Ipswich, Massachusetts*

C. Heiner, *Pacific Biociences, Menlo Park, California*

**Session VII: Human Variation and Disease**

R. Waterston, *University of Washington, Seattle*: *C. elegans*: How complete can we get?

H. Yang, *Beijing Genomics Institute, China*: China: A latecomer to the global sequencing effort.


M. Gerstein, *Yale University, New Haven, Connecticut*: ENCODE.


J. Lupski, *Baylor College of Medicine, Houston, Texas*: Applications of sequencing in clinical genetics.
Metabolic Signaling and Disease: From Cell to Organism

August 11–15 126 Participants

ARRANGED BY Daniel Kelly, Sanford-Burnham Medical Research Institute, Orlando, Florida
Mitchell Lazar, University of Pennsylvania, Philadelphia
Susanne Mandrup, University of Southern Denmark, Odense

This was the second meeting following a highly successful Metabolic Signaling and Disease meeting 2 years earlier. The main goal of this 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 organismal physiology and disease, including organ–organ cross-talk. 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. Stimulating keynote addresses were given by Diane Mathis and Daniel Rader, setting a superb tone on the opening evening. A total of 10 sessions followed, all highlighting unpublished research. Eight sessions featured oral presentations. The oral presentation sessions focused on key areas in the field of metabolism, including transcriptional and epigenomic control, receptor-mediated control, lipid flux and storage, and mitochondrial function. Following these topics, two sessions covered selected aspects of integrated metabolic signaling with emphasis on energy balance, organ–organ cross-talk, and the metabolic origins of aging and disease. The final session featured a current view of metabolism and cancer. 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. In all, there were 36 talks, by speakers from Canada, Europe, and Japan in addition to the United States. Twelve of the talks were given by women.
The total number of abstracts was 89, including 49 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, the interactive nature of the meeting led to stimulating questions and discussion that exposed attendees 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 Addresses
D. Mathis, Harvard Medical School, Boston, Massachusetts
D. Rader, University of Pennsylvania, Philadelphia

Transcriptional Networks Regulating Metabolism
Chairperson: M. Lazar, University of Pennsylvania, Philadelphia

Epigenomic Regulation of Metabolism
Chairperson: A. Kralli, Scripps Research Institute, La Jolla, California

Receptors in Metabolic Signaling
Chairperson: C. Glass, University of California, San Diego

Lipid Storage and Flux
Chairperson: B. Spiegelman, Dana-Farber Cancer Institute, Boston, Massachusetts

Regulation of Mitochondrial Function
Chairperson: B. Cannon, Stockholm University, Sweden

Integrating Metabolic Signaling I
Chairperson: O. MacDougald, University of Michigan, Ann Arbor

Integrating Metabolic Signaling II
Chairperson: D. James, University of Sydney, Australia

Metabolic Regulation in Stem Cells and Cancer
Chairperson: D. Moore, Baylor College of Medicine, Houston, Texas
This ninth meeting was held this summer to present and discuss recent developments in mRNA metabolism in eukaryotes. As in the past, the regulation of alternative pre-mRNA splicing was a focus, as was the mechanism of spliceosome assembly and catalysis. Important developments in genome-wide approaches were exemplified by several talks from the ENCODE project. This focus was dramatically illustrated on the cover of the abstract book with images of RNA-binding proteins presented in the form of a periodic table. Other topics included mRNA 3′-end formation, mRNA decay, mRNA trafficking, and the coupling of RNA processing to transcription, decay, and export. Structural studies, single-molecule imaging and live-cell imaging were interwoven into the sessions, reflecting state-of-the-art techniques currently applied to understanding how mRNA is processed in vivo and in vitro. Talks related to microRNAs, long noncoding RNAs, RNA modifications, virus-encoded RNAs, and the molecular basis of RNA-related diseases were also well represented.

The meeting began with a strong session in which global analyses of RNA processing were presented, followed by talks on novel RNA modifications. Highlights included the identification of new developmentally regulated alternatively spliced transcript isoforms, the discovery of isoform-specific subcellular localization signals that are developmentally regulated, the description of high-throughput analysis of RNA-binding proteins and their targets, and a presentation of the concept of RNA epigenetics (also known as epitranscriptome) facilitated by high-resolution mapping of modified nucleotides.

The splicing mechanisms session included a presentation on adaptation of the state-of-the-art method of colocalization single-molecule spectroscopy (CoSMoS) to splicing in human splicing extracts. Studies in yeast also provided a wealth of new insights into rearrangements and interactions within the spliceosome core, including an unexpected intermediate in the unwinding of U4/U6 snRNA duplex, and demonstration that Cwc25 modulates the catalytic state of the
spliceosome, controlling directionality of the splicing process. Talks in the alternative splicing and RNP sessions identified unknown RNA-binding proteins and provided insight into the regulation of RNA-binding proteins and how they control splicing under different cell growth conditions. Alternative polyadenylation was also featured, with several talks focusing on changes in polyadenylation in response to DNA damage and disease and links between polyadenylation and mRNA degradation.

In sessions on RNA turnover, translation, and small RNAs, several presentations focused on the variety of cofactors recruited by RNA-binding proteins to promote repression and turnover of mRNAs and their links to development and disease. Another major focus was on nuclear and cytoplasmic RNA quality-control pathways, including several presentations on recently discovered pathways that regulate the translation and stability of mRNAs according to characteristics of their coding regions. A further highlight was a presentation on a class of virus-encoded small RNAs that serve to recruit repressive factors to host cell mRNAs through base-pairing.

Finally, there was an exciting session on functional coupling of cotranscriptional splicing with other nuclear processes. A novel method to measure the kinetics of splicing individual transcripts relative to the progress of their transcription revealed that the spliceosome and RNA polymerase are in very close proximity when splicing occurs. In a complementary study, labeling of nascent transcripts followed by RNA-Seq permitted analysis of how gene features may affect the kinetics of splicing individual introns and the consequences for alternative splicing. The meeting concluded with several talks presenting further compelling evidence for functional links between splicing, transcription, 3′-end formation, DNA modification, DNA repair, and chromatin factors. The influence of RNA in multiple cellular processes continues to grow!

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health; the National Science Foundation; and Lexogen, Inc.

**PROGRAM**

**Global Analysis of RNA Processing/RNA Modification and Structure**  
Chairpersons: C. Burge, Massachusetts Institute of Technology, Cambridge; T. Pan, University of Chicago, Illinois

**Mechanisms of RNA Splicing**  
Chairpersons: S.-C. Cheng, Academia Sinica, Taipei, Taiwan; M. Moore, HHMI/University of Massachusetts Medical School, Worcester
RNA Turnover and Quality Control  
Chairpersons: T. Heick Jensen, Aarhus University, Denmark; J. Coller, Case Western Reserve University, Cleveland, Ohio

RNP Structure/RNP Systems Biology  
Chairpersons: S. Butcher, University of Wisconsin, Madison; J. Ule, University College London, United Kingdom

Viruses and RNA Processing/Small RNA Biogenesis and Function  
Chairpersons: B. Glaunsinger, University of California, Berkeley; E. Izaurralde, Max-Planck Institute for Developmental Biology, Tübingen, Germany

Alternative Splicing and Regulation  
Chairpersons: D. Black, HHMI/University of California, Los Angeles; J. Caceres, MRC Human Genetics Unit, Edinburgh, United Kingdom

3' Regulation/Translation  
Chairpersons: J. Manley, Columbia University, New York; W. Gilbert, Massachusetts Institute of Technology, Cambridge

Cotranscriptional RNA Processing/Coupling RNA Processing and Chromatin  
Chairpersons: H. Lou, Case Western Reserve University, Cleveland, Ohio; S. Oberdoerffer, National Cancer Institute, National Institutes of Health, Bethesda, Maryland
Mechanisms of Eukaryotic Transcription

August 25–29 314 Participants

ARRANGED BY Patrick Cramer, Max-Planck Institute of Biophysical Chemistry, Göttingen, Germany
Katherine A. Jones, The Salk Institute for Biological Studies, La Jolla, California
John Lis, Cornell University, Ithaca, New York

Regulation of gene transcription plays 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 functional genomics. This meeting appropriately covered many aspects of the field and brought together a diverse group of scientists; it comprised eight plenary sessions and two poster sessions.

The meeting began with a session that included exciting new insights derived from structure and imaging studies of human and yeast RNA polymerase preinitiation complexes as well as a genome-wide study of unstable nucleosomes on promoters. The second session covered new imaging technologies that revealed dynamics of transcription factor binding as well as studies of mechanistic interplay of transcription factors, coactivators, and chromatin in the regulation of transcription. The next plenary session (fourth session) described new views of long-range interactions of regulatory elements and promoters. The following session revealed new insights into the regulation that occurs broadly at promoter-proximal pause release and the role of factors in this and other steps during transcription elongation. The next plenary session (seventh session) provided further insight to Pol II pausing and broader mechanisms of regulation and their connection to disease. The eighth session continued with the elongation theme but was primarily focused on enzymatic complexes that bind and/or modify the RNAPII carboxy-terminal domain (CTD). A comprehensive identification of CTD phosphorylation and the players involved by mass spectrometry was of particular interest. The ninth session covered the mechanisms of chromatin associated complexes in transcriptional repression. The final session was an eclectic and interesting series of presentations on interplay of the transcriptome—in particular, noncoding RNAs— with gene regulatory processes.

Interspersed with these oral presentations were the two poster sessions where a wide variety of exciting unpublished transcriptional research was presented.

This meeting was funded in part by the National Science Foundation and Lexogen, Inc.
PROGRAM

Initiation Complexes
Chairperson: S. Hahn, Fred Hutchinson Cancer Research Center, Seattle, Washington

Transcription Factors and Interacting Coregulators
Chairperson: S. Buratowski, Harvard Medical School, Boston, Massachusetts

Enhancers and Long-Range Interactions
Chairperson: J. Mellor, University of Oxford, United Kingdom

Early Elongation and Regulatory Factors
Chairperson: K. Adelman, NIEHS, Research Triangle Park, North Carolina

Signaling and Regulation
Chairperson: K. Arndt, University of Pittsburgh, Pennsylvania

The CTD and Cotranscriptional Events
Chairperson: P. Cramer, Max-Planck Institute for Biophysical Chemistry, Germany

Chromatin Interplay with Transcription
Chairperson: K. Jones, The Salk Institute for Biological Studies, La Jolla, California

Shaping the Transcriptome
Chairperson: J. Lis, Cornell University, Ithaca, New York
Eukaryotic DNA Replication and Genome Maintenance

September 1–6  313 Participants

ARRANGED BY  Anne Donaldson, University of Aberdeen, United Kingdom
               Anindya Dutta, University of Virginia, Charlottesville
               Johannes Walter, Harvard Medical School, Boston, Massachusetts

This meeting provided a pivotal opportunity for discussion and exchange of ideas in the rapidly advancing field of chromosome duplication and genome stability research. Delegates attended from 22 different countries, reflecting the diversity, quality, and breadth of this research area, with investigators keen to share and discuss the latest breakthroughs. Approximately half of the 313 attendees were Ph.D. students or postdoctoral scientists. The number and diversity of attendees can be credited to this meeting’s established position as the foremost conference in the field of DNA replication. There were 80 platform talks grouped in eight sessions, with a total of 250 platform and poster presentations. Every one of the talks drew stimulating questions from the audience and provoked ongoing discussion continuing into the social parts of the program. The poster sessions were packed throughout and distinguished by lively discussion, with interactions encouraged by the welcoming design of the new Nicholls-Biondi poster pavilion.

The ten platform sessions listed below were arranged in order of cellular events. Early sessions described the identification and functional requirements of replication initiation sites in mammalian cells and outlined recent structural information on protein complexes central to DNA replication. Major conceptual advances are being provided by the recent establishment of in vitro systems recapitulating central processes of DNA replication, including origin initiation, replication termination, and aspects of DNA repair. One important theme of the meeting was the insights obtained from these systems into the biochemistry of replisome assembly and replication termination, and how replication proteins also contribute to DNA repair.

The organizers continued their strategy of broadening the conference scope to encompass the extensive intersect of replication processes with genome stability mechanisms. It was therefore pleasing that the final two sessions of the meeting were particular highlights, covering physiological effects of derailed DNA replication and with several talks describing the use of cutting-edge genomic and proteomic technologies to uncover pathways that protect fragile replicating DNA against potentially carcinogenic changes. Overall, the meeting provided an excellent update on current understanding, with the broad range and high quality of scientific advance and discussion fully confirming this as the preeminent conference in this fast-moving and dynamic research field.
The meeting depended on funding provided by the National Cancer Institute, a branch of the National Institutes of Health.

**PROGRAM**

**Origin Specification**
*Chairpersons:* I. Whitehouse, Sloan Kettering Institute, New York; M.-N. Prioleau, CNRS, Université Paris Diderot, France

**Helicase Loading, Activation, and Timing Control**
*Chairpersons:* S. Bell, Indiana University, Bloomington; C. Speck, Imperial College London, United Kingdom

**Effect of Chromatin and Transcription Factors on Replication**
*Chairpersons:* F. Chedin, University of California, Davis; K. Cimprich, Stanford University, California

**Posttranslational Modifications**
*Chairpersons:* J. Cook, University of North Carolina, Chapel Hill; H. Masai, Tokyo Metropolitan Institute of Medical Science, Japan

**Elongation Phase**
*Chairpersons:* L. Pellegrini, University of Cambridge, United Kingdom; S. Benkovic, Pennsylvania State University, University Park

**Obstacles, DNA Damage, and Genome Instability**
*Chairpersons:* V. Zakian, Princeton University, New Jersey; I. Hickson, University of Copenhagen, Denmark

**The Response to Replication Stress**
*Chairpersons:* A. Malkova, University of Iowa, Iowa City; L. Zou, Harvard Medical School, Boston, Massachusetts

**Cancer, Disease, and Development**
*Chairpersons:* O. Fernandez-Capetillo, Spanish National Cancer Research Centre, Madrid; S. Mirkin, Tufts University, Medford, Massachusetts
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 host response and immunity, the contribution of determinants in individual cells to host–microbe interactions, commensalism, dysbiosis and pathogenesis, microbial surfaces and effectors, metabolism, structure, and evolution of virulence, and regulation and signaling of virulence. 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. Jorge Galan presented the keynote address. Dr. Galan is an internationally recognized leader in the field of biomedical research.
His presentation, Pathogen Restriction and Host Specificity or Why *Salmonella typhi* Can Only Infect Humans, provided an exciting historic overview of the biology of typhoid fever and the science behind our current understanding of the unique characteristics of this key human pathogen.

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 poster sessions, and continued during a wine and cheese reception and other social gatherings. We strongly encouraged submission of abstracts by junior researchers in the field, and many young investigators were in attendance. Some of these interactions have already produced fruitful scientific collaborations.

This meeting was partly supported by funds from the National Institute of Dental and Craniofacial Research and the National Institute of Allergy and Infectious Diseases.

**PROGRAM**

**Host Response and Immunity**
*Chairperson: M Netea, Radboud University, Nijmegen, The Netherlands*

**The Individual vs. the Community: The Contribution of Determinants in Individual Cells to Host–Microbe Interactions**
*Chairperson: E. Rubin, Harvard University, Boston, Massachusetts*

**Commensalism, Dysbiosis, and Pathogenesis**
*Chairperson: L. Hooper, University of Texas Southwestern Medical Center, Dallas*

**Microbial Surfaces and Effectors**
*Chairperson: V. Nizet, University of California, San Diego*

**Metabolism, Structure, Evolution, and Virulence**
*Chairperson: A. Brown, University of Aberdeen, United Kingdom*

**Regulation and Signaling of Virulence**
*Chairperson: A. Camilli, Tufts University, Boston, Massachusetts*

**Keynote Address**
J. Galan, Yale University, New Haven, Connecticut
Cell Death

September 15–19 187 participants
ARRANGED BY David Andrews, University of Toronto, Canada
Douglas Green, St. Jude Children’s Research Hospital, Memphis, Tennessee
Anthony Letai, Dana-Farber Cancer Institute, Boston, Massachusetts

Regarded by many present as perhaps the best cell death meeting in years, more than 136 outstanding presentations and lively discussion centered on topics in the field of cell death.

There were two keynote lectures. The first was given by Andreas Strasser on Turning Discoveries on Cell Death into Novel Cancer Therapies, a very topical presentation given the imminent approval of venetoclax for use in human cancers. The meeting further elaborated this theme with a session dedicated to the discovery and validation of small molecule tool compounds that modulate different Bcl-2 family proteins. The second was given by Seamus Martin on danger signals and the role of interleukin 1β and other cytokines in immune regulation of cell death. This keynote put the spotlight on an active and highly controversial topic in cell death. Other highlights included presentations by Peter Czabotar on structural insights into the activation of Bax and Bak, Peter Sorger on measuring and modeling molecular mechanisms of apoptosis, Sandra Zinkel on how Bid regulates apoptosis/necroptosis and cell death fate in hematopoiesis, Laura Attardi on deconstructing p53 tumor suppression pathways in vivo, Simone Fulda on novel opportunities to target IAP proteins for cancer therapy, and Kim Newton on regulation of cell death by the kinase RIPK1. Approximately a third of the presentations were given by women, which reflected well the overall composition of the meeting.

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, as well as more productive in terms of exploration of new ideas.

A total of 86 posters were also presented in two sessions, one held traditionally in the afternoon prior to the wine and cheese reception and a second held during the morning. This was the second time that a morning poster session attracted active discussion and very enthusiastic attendance, suggesting that this approach should be continued in future meetings.

It is not surprising that 20 years after its “rebirth” in the early ’90s, the field of Cell Death has advanced enormously and is generating tremendous excitement in areas such as structural biology, translational research, and clinical impact. Moreover, it is well recognized that cell
death is a fundamental biological process regulated by several partly parallel pathways. As a result, new insights into its mechanisms unveil new processes and phenomena with deep implications for biology. The imminent FDA approval of therapeutics that control cell death is expanding the reach of the field to the clinic with amazing results. As we heard at the meeting, regulation of apoptosis with drugs is beginning to impact precision medicine. The next meeting will be even better, we are sure.

This conference was funded in part by the National Institute on Aging and the National Cancer Institute, both branches of the National Institutes of Health.

PROGRAM

Keynote Addresses
A. Strasser, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia
S. Martin, Trinity College Dublin, Ireland

BCL-2 Family Proteins I
Chairperson: P. Czabotar, Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia

Regulated Necrosis
Chairpersons: W. Kaiser, Emory University School of Medicine, Atlanta, Georgia; A. Linkermann, University of Kiel, Germany

Non-Apoptotic, Non-Necroptotic Cell Death
Chairpersons: B. Stockwell, Columbia University, New York; A. Thorburn, University of Colorado, Aurora

BCL-2 Family II
Chairperson: J. Chipuk, Icahn School of Medicine at Mount Sinai, New York

Metabolic Control of Cell Death
Chairpersons: E. Gottlieb, Cancer Research UK, Glasgow, United Kingdom; L. Attardi, Stanford University, California

Molecular Mechanisms I
Chairperson: M. Bogyo, Stanford University, California

Cell Death Therapeutics
Chairpersons: S. Fulda, Goethe-Universität Frankfurt, Germany; L. Boise, Emory University, Atlanta, Georgia

Cell Death and Inflammation
Chairpersons: K. Newton, Genentech, Inc., South San Francisco, California; T.-D. Kanneganti, St. Jude Children’s Research Hospital, Memphis, Tennessee

Molecular Mechanisms II
Chairperson: D. Andrews, University of Toronto, Canada

Cell Death in Cancer Biology
Chairpersons: M. Yaffe, Massachusetts Institute of Technology/Broad Institute, Cambridge; G. Evan, University of Cambridge, United Kingdom
Genome Engineering: The CRISPR/Cas Revolution

September 24–27    398 Participants

ARRANGED BY    Jennifer Doudna, HHMI/University of California, Berkeley
                Maria Jasin, Memorial Sloan Kettering Cancer Center, New York
                Jonathan Weissman, HHMI/University of California, San Francisco

This was the first time this meeting was held at Cold Spring Harbor. Genome engineering, as accomplished through the introduction of breaks in the DNA backbone, has been possible for several years. However, the recent discovery of bacterial adaptive immunity and the co-opting of the Cas9 component from it have led to the "democratization" of genome engineering, such that scientists working in many research areas and organisms can readily utilize the approach. Furthermore, Cas9 allows multiplexing to an extent that was not possible with previous approaches, and it is readily adapted for control of transcription and other applications, which were also highlighted at the conference.

A goal of this meeting was to bring together researchers working in diverse fields to stimulate discussions and ideas to further exploit Cas9 and related technologies for biological discovery and medical applications. Twenty speakers were invited to cover these diverse topics. Another 38 speakers were chosen from submitted abstracts. Presenters represented institutions from the United States and abroad (Germany, United Kingdom, the Netherlands, Australia, Korea, Belgium, Saudi Arabia, and China) and also included three representatives from industry. Speakers chosen from submitted abstracts included lab heads/staff scientists and postdoctoral fellows. Approximately 115 posters were presented in two sessions, complementing the oral presentations.

The first talk of the meeting by Emmanuelle Charpentier introduced the CRISPR/Cas9 system in bacteria; Jennifer Doudna, filling in for the last-minute cancellation by George Church, spoke on the last day of the meeting, highlighting recent results from her lab involving structural biology and biophysics of CRISPR components. Other talks utilized molecular, cell, and computational biology in diverse model organisms, as well as economically important and some unconventional organisms. Much of the data presented was unpublished or only very recently published.

A panel discussion was also included focusing on the ethics of human genome germline editing. The panel included the three organizers as well as ethicist Hank Greely and Fyodor Urnov, an employee of Sangamo BioSciences, which has been a leader in using genome engineering in the clinic. The panel discussion was well attended by the meeting attendees, who participated heavily in the discussion with questions and comments.

This meeting was funded in part by Advanced Analytical; Clontech Laboratories; Diagenode; Editas Medicine; and Thermo Fisher Scientific.
PROGRAM

CRISPR
Chairpersons: E. Charpentier, Max-Planck Institute for Infection Biology, Berlin, Germany; F. Zhang, Massachusetts Institute of Technology, Cambridge

Stem Cells and Human Disease
Chairpersons: D. Huangfu, Memorial Sloan Kettering Cancer Center, New York; B. Conklin, Gladstone Institute of Cardiovascular Disease, University of California, San Francisco

Discussion on Ethics of Human Germline Genome Editing
Chairpersons: J. Doudna, HHMI/University of California, Berkeley; M. Jasin, Memorial Sloan Kettering Cancer Center, New York; J. Weissman, HHMI/University of California, San Francisco

Mouse Models and Cancer
Chairpersons: A. Ventura, Memorial Sloan Kettering Cancer Center, New York; W. Skarnes, Wellcome Trust Sanger Institute, Hinxton, United Kingdom

DNA Repair
Chairpersons: L. Symington, Columbia University, New York; N. Maizels, University of Washington, Seattle

Model Organisms
Chairpersons: J.-S. Kim, Seoul National University, Korea; J.K. Joung, Massachusetts General Hospital, Charlestown

Technology
Chairpersons: S. Qi, Stanford University, California; B. Huang, University of California, San Francisco
This meeting focused on stimulating discussion of new ideas, techniques, and discoveries in Drosophila neurobiology. Eleven sessions ran in series over 4 days, with alternating platform and poster presentations plus a workshop session with multiple concurrent breakout groups that emphasized an interactive format. Alternating between the more and less formal presentations provided excellent opportunities to meet other attendees and discuss scientific issues one-on-one. These interactions were excellent for facilitating collaborations, exchange of reagents (e.g., antibodies, clones, mutants, and other stocks), methods (genetic, physiological, optical), and ideas between both new and established investigators. The seven platform session topics were chosen to reflect the areas in which cutting-edge advances are being made. Session chairs and the meeting organizers selected presenters for these platform sessions from the submitted abstracts, and the remaining abstracts were presented as posters. The vast majority of the speakers were graduate students and postdoctoral fellows, and approximately half were female. Many people commented positively on the addition of a glial biology session, a topic not represented in previous years. In the keynote address, the Benzer lecture, Leslie Griffith presented a stimulating presentation on the molecular, cellular, and circuit mechanisms underlying sleep. The Elkins Award plenary lecture is presented at each meeting by a graduate student whose dissertation exemplifies the finest work in our field. This year the Elkins lecture was presented by Dr. Pip Coen, who trained as a graduate student with Dr. Mala Murthy at Princeton University, and who presented his spectacular work on acoustic communication in Drosophila. New this year were concurrent workshop sessions held on Thursday morning. Some were large, and others small, but they all involved interactive components.
that stimulated lively discussion and interaction among the participants. A straw poll conducted on the last day indicated overwhelming support for including a workshop session in future meetings. A career mentoring dinner was also a huge success, with faculty volunteering to host tables for about 150 graduate students and postdocs covering a variety of topics related to professional development. This was the best-attended Neurobiology of Drosophila meeting in history, with presentations spanning the breadth of modern neurobiology. The many opportunities for interaction and career development fostered by this meeting are sure to enhance this vibrant 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: M. Wu, Johns Hopkins University, Baltimore, Maryland*

**Neural Development**
*Chairperson: H. Broihier, Case Western Reserve University, Cleveland, Ohio*

**Sensory Systems**
*Chairperson: T. Holmes, University of California, Irvine*

**Elkins Memorial Lecture**
P. Coen, University College London, United Kingdom

**WORKSHOPS**

**Glial Biology**
*Chairperson: F. Rob Jackson, Tufts University School of Medicine, Boston, Massachusetts*

**Seymour Benzer Lecture**
L. Griffith, Brandeis University, Boston, Massachusetts

**Mechanisms of Neurological Diseases**
*Chairperson: M. Guo, University of California, Los Angeles*

**Synaptic Transmission and Plasticity**
*Chairperson: R. Hiesinger, Freie Universität Berlin, Germany*

**Neural Circuits and Higher-Order Nervous System Function**
*Chairperson: K. Scott, University of California, Berkeley*
This fourth biannual conference was organized into thematic topics, including pluripotency control, mechanisms of reprogramming, stem cells and epigenetics, tissue stem cells and regeneration, therapy with stem cells, and neural stem cells. Approximately 31% of participants were Ph.D. students, whereas 22% were postdocs from 25 different countries. Representatives from major publishing houses and publications were present such as Cell Press, Nature Communications, and Development. Speakers comprised a mix of internationally known leaders in the individual disciplines as well as emerging junior researchers, who presented their recent work. In addition, 18 short talks were chosen from abstracts.

Main talks were 20–25 minutes long plus 5–10 minutes of discussion, whereas short talks were 10 minutes plus 5 minutes of discussion. The keynote lecture was given by Elly Tanaka, who presented exciting unpublished work on factors controlling axolotl regeneration. Most of the presented work was unpublished and led to stimulating discussions immediately after the talk, during poster sessions and meals, and 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 fact that most speakers stayed for several days and were approachable throughout the conference.

This meeting was funded in part by Clontech and Fluidigm.
Meetings

PROGRAM

Control of Pluripotency
Chairperson: K. Hochedlinger, Harvard Medical School/Massachusetts General Hospital, Boston

Cellular Reprogramming
Chairperson: J. Hanna, Weizmann Institute of Science, Rehovot, Israel

Stem Cells and Epigenetics
Chairperson: H. Blau, Stanford University School of Medicine, California

Tissue Stem Cells and Regeneration
Chairperson: K. Zaret, University of Pennsylvania Perelman School of Medicine, Philadelphia

Therapy with Stem Cells
Chairperson: F. Watt, King’s College London, United Kingdom

Neural Stem Cells
Chairperson: M. Wernig, Stanford University, California

Stem Cells and the Environment
Chairperson: B. Hogan, Duke University, Durham, North Carolina

Keynote Address
E. Tanaka, University of Dresden, Germany
Probabilistic Modeling in Genomics

October 14–17 211 Participants

ARRANGED BY Barbara Engelhardt, Princeton University, New Jersey
Thomas Mailund, Aarhus University, Denmark
Adam Siepel, Cold Spring Harbor Laboratory

This was the first conference hosted at Cold Spring Harbor, following workshops on a similar topic in 2013 at Janelia Farm and in 2014 at Merton College, Oxford. The main goal of this meeting was to provide a forum for presentation and exchange of ideas among researchers who are working in the general area of genomics, but are particularly focused on the development of new probabilistic models, algorithms, and methods for inference. These researchers come from a variety of backgrounds, including computer science, statistics, applied mathematics, and physics. Previous workshops on this topic have strongly emphasized population genetics, but the program this time was designed to incorporate additional areas of interest such as functional genomics, systems biology, and quantitative genetics. Session topics presented at the meeting are listed below. There were six oral presentations per session for a total of 36 presentations. Two invited session chairs presented in each session, and four additional talks were selected from submitted abstracts. In addition, three of the session chairs opted to pass on their speaking slots, so a total of 27 talks went to people who submitted abstracts, the vast majority of whom were graduate students and postdocs. All talks were 20 minutes long plus 5 minutes for questions and answers. The quality of the presentations was very high overall, with considerable mathematical sophistication but also an eye toward biological relevance.

We were honored to recruit two distinguished senior scientists to attend the meeting and present keynote lectures: Michael Jordan and Elizabeth Thompson. Jordan gave a wide-ranging, entertaining, and thought-provoking lecture on the field of Nonparametric Bayesian Statistics,
making the argument that these methods deserve broader application in computational biology. Thompson gave a wonderfully thoughtful lecture on methods for modeling and inferring co-ancestry, particularly tracts of identity by descent (IBD). Her lecture tied together a number of influential ideas she has developed during her decades of work on this general topic. The two keynote lectures complemented each other nicely, one being broader and coming from someone outside the field (Jordan) and the other more technical and coming from a highly respected insider (Thompson).

In general, the meeting was characterized by a great deal of enthusiastic discussion and a collaborative and collegial atmosphere. Many attendees commented on a desire to establish PROBGEN as an annual event, perhaps alternating between Cold Spring Harbor and a venue in Europe. Discussion is under way for a meeting at Oxford in 2016.

**PROGRAM**

**Demography and Admixture**  
*Chairpersons: J. Novembre, University of Chicago, Illinois; Y. Song, University of California, Berkeley/University of Pennsylvania, Philadelphia*

**Assembly and Variant Identification**  
*Chairpersons: R. Durbin, Wellcome Trust Sanger Institute, Hinxton, United Kingdom; D. Haussler, HHMI/University of California, Santa Cruz*
Keynote Address
M.I. Jordan, *University of California, Berkeley*

**Systems and Structural Biology**
*Chairpersons:* M. Singh, Princeton University, New Jersey;
O. Troyanskaya, Princeton University, New Jersey

**Population Genetics and Natural Selection**
*Chairpersons:* R. Nielsen, University of California, Berkeley;
A. Williams, Cornell University, Ithaca, New York

**Functional Genomics**
*Chairpersons:* L. Pachter, University of California, Berkeley;
S. Richardson, MRC Biotatistics Unit, Cambridge,
United Kingdom

Keynote Address
E.A. Thompson, *University of Washington, Seattle*

**Quantitative Genetics**
*Chairpersons:* J. Listgarten, Microsoft Research New England,
Cambridge, Massachusetts; M. Stephens, University of
Chicago, Illinois
This 13th conference series, which was held at CSHL this year, continues to demonstrate a robust attendance, both from the United States and from abroad. It attracted more than 300 registrants, presenting more than 200 abstracts, that 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, and we also had 166 posters presented.

The 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 topics featured at the sessions are listed below.

The first keynote address was delivered by Dr. Aviv Regev, who focused on the innovative application of single-cell sequencing to the understanding of tumor evolution and metastasis. The second keynote was delivered by Dr. Mark Gerstein, who focused on novel methods for large-scale transcriptome mining—especially methods for building integrative regulatory models while protecting individual privacy. Both talks were extremely well regarded, with much follow-up discussion.

This meeting was very actively discussed on Twitter (using hashtag #gi2015), with the social media policy being opt-out (with virtually all talks being tweetable). More than 2000 messages were broadcast 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. The poster competition was sponsored by *Genome Research*.

**PROGRAM**

**Personal and Medical Genomics**  
*Chairpersons:* D. Church, Personalis, Inc., Menlo Park, California; K. Meltz Steinberg, Washington University, St. Louis, Missouri

**Transcriptomics, Alternative Splicing, and Gene Predictions**  
*Chairpersons:* C. Trapnell, University of Washington, Seattle; A. Battle, Johns Hopkins University, Baltimore, Maryland

**Keynote Address**  
A. Regev, Broad Institute, Cambridge, Massachusetts

**Epigenomics and Noncoding Genome**  
*Chairpersons:* M. Gymrek, Harvard University/Massachusetts Institute of Technology Division of Health Sciences and Technology, Cambridge; M. Hoffman, University of Toronto, Canada

**Databases, Data Mining, Visualization, Ontologies, and Curation**  
*Chairpersons:* S. Lewis, Lawrence Berkeley National Laboratory, California; J. Aerts, KU Leuven, Belgium

**Sequencing Pipelines and Assembly**  
*Chairpersons:* G. Lunter, University of Oxford, United Kingdom; A. Quinlan, University of Utah, Salt Lake City

**Keynote Address**  
M. Gerstein, Yale University, New Haven, Connecticut

**Comparative, Evolutionary, and Metagenomics**  
*Chairpersons:* A. McLysaght, Trinity College Dublin, Ireland; A. Siepel, Cold Spring Harbor Laboratory
The 2015 meeting continued the successful partnership of bringing together cell biology of both budding (Saccharomyces) and fission (Schizosaccharomyces) yeasts, as well as related organisms.

This year featured for the first time a keynote speaker, Susan Lindquist, who described amazing progress enabled by using yeast to understand and ameliorate the toxicity caused by misfolded proteins in neurodegenerative diseases such as Parkinson’s and Alzheimer’s. Intriguingly, several selected talks described advances indicating that protein aggregation by intrinsically disordered domains can also play positive roles in organismal biology. Andrew Capaldi showed that aggregation of the TOR signaling complex helps commit cells to a quiescent state under low-nutrient conditions, whereas Shady Saad (Matthias Peter lab) showed that the metabolic enzyme pyruvate kinase undergoes regulated aggregation in response to nutrient and other stresses. Such modulations of protein conformation represent new links between nutritional sensing/signaling and cell-cycle control. More broadly, Daniel Jarosz described large-scale screens identifying nearly 50 yeast proteins whose transient overexpression leads to long-term epigenetic changes. Interestingly, many of the relevant proteins do not contain prion-like domains and do not form large aggregates, but nearly all are strongly enriched for intrinsically disordered domains.

The exploitation of disordered domains within proteins to evolve sophisticated regulatory codes was elucidated in several talks. Mart Loog described in exquisite biochemical detail how processive phosphorylation events in the CDK inhibitor Far1 (a disordered protein), together with a panoply of cyclin/CDK docking motifs, contributes to decision-making between mating and cell-cycle control. Eric Weiss described systems-level approaches to identifying short, linear docking motifs in substrates of a Hippo pathway kinase. These motifs, which increase kinase specificity and robustness, are present mostly within disordered domains, suggesting a means by which kinase-substrate specificity can evolve rapidly to generate diverse signaling networks in different organisms.

Structural (crystallography, EM) and biophysical approaches featured in several excellent talks, providing insights into BAR-domain function in cytokinesis (Nathan McDonald, Kathy Gould), septin oligomerization (Michael McMurray), ESCRT-III complex-driven membrane invagination (Steven Tang, Scott Emr), and the molecular architecture of the exocyst complex (Maggie Heider, Mary Munson). Trisha Davis described powerful new techniques to reveal conformational changes in multiprotein complexes by combining cross-linking mass spectrometry with constraint-based computational modeling.

We learned about new checkpoints responding to defects in vacuole inheritance (Lois Weissman) and septum formation (Jennifer Brace, Eric Weiss), and a new model to explain the long-standing puzzle of cell size control (Devon Chandler-Brown, Jan Skotheim). Francesc Posas elucidated how p38 stress-activated protein kinase coordinates stress-induced gene expression with...
S-phase arrest; Ken Sawin showed that stress-activated signaling directly inhibits cell polarity upon nutrient or drug perturbations; and Ray Deshaies described a new mechanism for detecting and eliminating excess ribosomal proteins, which constitute the predominant protein biosynthetic load in all cells.

Widening the meeting focus to other fungi yielded a comprehensive overview of the extraordinary remodeling of cell behavior enacted by circadian clocks in *Neurospora* (Jay Dunlap) and the discovery of a novel quorum sensing system required for virulence of the pathogenic yeast *Cryptococcus* (Christina Homer, Hiten Madhani).

One signal aspect of the meeting was the participation of graduate students—not only in giving excellent talks and poster presentations but also in asking (and handling) questions from the floor. All participants benefited from vigorous discussion of wide-ranging topics in cell biology in an exceptionally friendly and interactive atmosphere. Perhaps enabled by the relatively small size of the meeting and the improved facilities provided by the Nicholls-Biondi Hall venue for poster sessions, the quality of scientific exchange was consistently at the highest level.

This meeting was funded in part by the National Institute of General Medical Sciences, a branch of the National Institutes of Health; and the National Science Foundation.

**PROGRAM**

**Cell Cycle Control**
*Chairpersons:* M. Loog, University of Tartu, Estonia; S. Hauf, Virginia Tech, Blacksburg

**Polarity and Cytokinesis**
*Chairpersons:* K. Gould, Vanderbilt University, Nashville, Tennessee; I. Hagan, University of Manchester, United Kingdom

**Amazing Yeast**
*Chairperson:* S. Lindquist, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

**Keynote Address**
S. Lindquist, HHMI/Whitehead Institute for Biomedical Research, Massachusetts Institute of Technology, Cambridge

**Membrane Traffic**
*Chairpersons:* L. Weisman, University of Michigan, Ann Arbor; A. Brescher, Cornell University, Ithaca, New York

**Mating and Sporulation**
*Chairpersons:* S. Martin, University of Lausanne, Switzerland; J. Skotheim, Stanford University, California

**Stress Response and Quality Control**
*Chairpersons:* R. Deshaies, California Institute of Technology, Pasadena; M. Cyert, Stanford University, California

**Nucleus and Mitosis**
*Chairpersons:* Y. Hiraoka, Osaka University, Japan; A. Marston, University of Edinburgh, United Kingdom

**Signaling**
*Chairpersons:* F. Posas, Universitat Pompeu Fabra, Barcelona, Spain; J. Dunlap, Geisel School of Medicine at Dartmouth, Hanover, New Hampshire
Single-Cell Analysis

November 11–15  156 Participants

ARRANGED BY  Nancy Allbritton, University of North Carolina, Chapel Hill
Scott Fraser, University of Southern California, Los Angeles
Junhyong Kim, University of Pennsylvania, Philadelphia

The goal of this third meeting was to bring together scientists who work with single cells using different experimental paradigms to discuss the progress that is being made. Nearly 160 scientists convened with two keynote talks, 31 talks, and 54 posters. Many cell types were discussed including bacteria, *Caenorhabditis elegans*, and mammalian cells. The range of techniques that were used to investigate single-cell biology included single-cell dissection and transcriptomics, single-cell genomic DNA sequencing, proteomics, and metabolomics—all at single-cell resolution. A common theme from many of the talks and posters was individual cell-to-cell variability and how it relates to organismal function. Several talks discussed translational application of single-cell assays, and there was particular progress in the single-cell analysis of human cells discussed by several presenters. Several speakers presented novel molecular probes for individual cell function and methods to mechanically manipulate individual cell interactions. The importance of, and necessity for, microfabrication and nanotechnology was highlighted in several talks. There was continued effort at developing multimodal measurements at the single-cell level assisted by these technologies. The need for quantitative analysis of
biological properties/processes in live cells in their natural microenvironment and moving toward a comprehensive model of molecular cell function was a consistent theme. The analysis of single-cell biology will undoubtedly lead to a better understanding of disease state, better production of biofuels, and insight into evolutionary mechanics that is unachievable using pooled cells.

Major support for this meeting has been provided by Fluidigm. Additional support was provided by Cell Microsystems; Clontech; QIAGEN; and Zeiss.

PROGRAM

Keynote Address
J. Lippincott-Schwartz, National Institutes of Health, Bethesda, Maryland

Applications of Single-Cell Biology
Chairperson: T. Meyer, Stanford University, California

Limits of Measurement
Chairperson: J. Eberwine, University of Pennsylvania Perelman School of Medicine, Philadelphia

Technologies, Chemistries, and Platforms
Chairperson: N. Allbritton, University of North Carolina, Chapel Hill

Analysis Challenges
Chairperson: J. Kim, University of Pennsylvania, Philadelphia

NIH Questions and Answers: The Future of Single-Cell Analysis
Moderators: N. Allbritton, University of North Carolina, Chapel Hill
S. Fraser, University of Southern California, Los Angeles
J. Kim, University of Pennsylvania, Philadelphia

Keynote Address
J.V. Sweedler, University of Illinois, Urbana

Future Visions
Chairperson: S. Fraser, University of Southern California, Los Angeles
Behavior and Neurogenetics of Nonhuman Primates

November 18–21 29 Participants
ARRANGED BY Nelson Freimer, University of California, Los Angeles
                Jeffrey Rogers, Baylor College of Medicine, Houston, Texas

This second CSHL meeting was intended to bring together a diverse set of researchers working on various aspects of primate neurobiology and/or behavior, with particular focus on genetics as a bridging concept across fields. Although we continued this overall approach, this time, our speaker invitations focused on investigators studying gene expression and/or gene regulation as either drivers of behavioral variation or outcomes of neurodevelopmental mechanisms. The first session addressed issues concerning genetic influences on behavior, analyzed through comparative studies of populations across differing environments or through controlled experiments in pedigreed research colonies. Session two dealt primarily with recent advances in genetic technologies, including in-depth discussion of optogenetic manipulation of brain circuits and transgenic manipulations of brain genes. Dr. Katherine Pollard provided an outstanding keynote lecture in which she discussed the evolution of gene regulatory elements critical for understanding comparative neurodevelopment and brain evolution. Session three featured shorter talks from submitted abstracts and addressed methods for and/or results from analyses of polymorphisms among closely related but behaviorally distinct primate populations. Session four explored the relationship between genetic variation within species and selection pressures that either constrain that variability or drive long-term changes in it. Speakers presented studies of natural selection acting on patterns of gene expression, the functional characteristics of intraspecies sequence variation, and the heritability of anatomical variation in gyrification across the cerebral cortex. The presentations in session five returned to the theme of gene expression and the forces that influence differences in expression, at the level of either individual ontogeny or species-level divergence. Session six focused on aspects of gene function and its relationship to behavioral phenotypes, specifically studies of how genetic differences drive differences in response to stress or how manipulation of the social environment can drive changes in gene expression. These brief summaries do not capture all the issues and topics covered in these sessions, but they reflect the major themes shared among presentations. As with our first meeting, one goal was to assemble researchers working on related complementary...
questions who do not normally attend the same meetings. We were again successful; many attendees remarked privately or in the open discussion that capped the meeting that they benefited from hearing presentations that touched on their own research areas, but that pushed the boundaries of their knowledge and experience. Several participants indicated they expected to maintain contact with people they met here for the first time and were optimistic that new collaborations may follow. The participants encouraged future meetings on this general topic, following a similar organization.

This meeting was funded in part by the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health.

PROGRAM

Opening Session
L. Barrett, University of Lethbridge, Alberta, Canada

Technologies
Chairpersons: X.-J. Li, Emory University, Atlanta, Georgia; E. Boyden, Massachusetts Institute of Technology, Cambridge

Keynote Address and Short Talks
K. Pollard, University of California/J. Gladstone Institutes, San Francisco

Evolution II
Chairperson: W. Enard, Ludwig-Maximilians University Munich, Martinsried, Germany

Development and Gene Expression
Chairperson: N. Freimer, University of California, Los Angeles

Traits
Chairpersons: J. Capitanio, University of California, Davis; N. Kalin, University of Wisconsin, Madison
This 10th meeting demonstrated the power of genome-enabled plant biology in a broad spectrum of areas ranging from environmental adaptation to developmental network modeling to crop improvement. Participants and presenters were a healthy mix of junior and established scientists, staying true to the vision of the first meeting in 1997 and the spirit of Cold Spring Harbor Laboratory. A breadth and depth of exciting, new unpublished work toward discovery of fundamental principles and direct applications in agriculture was presented.

A major theme across sessions was that of disruptive tools being developed or applied to address technical challenges and answer key biological questions. For example, a few talks presented advances in sequencing technology, such as PacBio’s long-read technology, that are poised to usher in a new era of gold-standard plant genomes and will facilitate future molecular genetic studies in crops with large, complex genomes such as wheat and sugarcane.

Another major theme was that of disruptive concepts and out-of-the-box thinking. Examples included work aimed at determining the global landscape of RNA secondary structures and RNA–protein interactions, research on long-distance-acting transcriptional regulatory elements in plants, and efforts to assign molecular functions to the “dark matter” of genomes, which remains a problem even for the most intensively studied model organisms.

Another prominent thread in the meeting related to the rapid development and adoption of high-throughput phenotyping in model and crop plants. These new technologies and approaches are enabling the characterization of developmental processes at many scales and uncovering of genetic variation underlying potentially useful traits. Talks presented open-source image analysis...
software suites for aboveground plant phenotyping, as well as development of imaging tools to quantify root architectural and physiological traits and measure the degree of plasticity in root growth dynamics in response to changing nutrient availability. These and other studies described at the meeting emphasize the multidisciplinary nature of genome-enabled quantitative biology to harness new phenotypic variation and genetic epistasis for crop improvement. Descriptions of state-of-the-art, high-throughput technologies and genome biology were complemented nicely by presentations on genetic, molecular, and spatial dissections of biological processes in model and crop species.

The keynote address was presented by Dr. John Doebley. His talk focused on his pioneering work in maize and teosinte that has elucidated several key genes and pathways critical for the domestication of maize from its ancient wild progenitor. Dr. Doebley’s talk was well received and generated numerous questions and an active follow-up discussion.

This meeting was funded in part by the National Science Foundation.

PROGRAM

Biodiversity/Environmental Adaptation
Chairperson: T. Mitchell-Olds, Duke University, Durham, North Carolina

Tool Development/New Frontiers
Chairperson: J.T. Cuperus, University of Washington, Seattle

Complex Traits/Synthetic Biology/Biotech
Chairperson: G. Coupland, Max-Planck Institute for Plant Breeding Research, Köln, Germany

Genetics/Epigenetics/Genome Biology
Chairperson: J. Law, The Salk Institute for Biological Studies, La Jolla, California

Development Networks/Modeling
Chairperson: S. Rhee, Carnegie Institute of Science, Stanford, California

Biotic/Abiotic Stress Signaling
Chairperson: T. Kroj, INRA-Montpellier, France

Keynote Speaker
Chairperson: J. Doebley, University of Wisconsin, Madison

Crop Genomics/Improvement
Chairperson: B. Keller, University of Zurich, Switzerland

D. Jackson, Y. Helariutta

Y. Liu
Rat Genomics and Models

December 9–12 69 Participants
ARRANGED BY Aron Geurts, Medical College of Wisconsin, Milwaukee
Michael Gould, University of Wisconsin-Madison
Bina Joe, The University of Toledo, Ohio
Enrico Petretto, Imperial College London, United Kingdom

This ninth biannual winter biotechnology conference has been held since 1999 to focus principally on the unique physiological and genetic relevance of the rat as a model organism for human diseases. A complementary meeting outside of the United States is held in the alternating years (Hinxton, United Kingdom 2014; Cambridge, United Kingdom 2012; Kyoto, Japan 2010). The primary goals of this meeting were (1) to promote interactions between biomedical researchers using rat models in the study of emerging technology, genomics, systems-biology, physiology, pathophysiology, metabolism, neuroscience, and oncology; (2) to provide opportunity for young investigators and trainees to network with leaders in the rat genomics community; and (3) to provide an interface between the research community and the entities that support biomedical research in which rat models are utilized.

Eight scientific sessions featured both invited speakers and selected abstracts. Opening keynote lecturer Dr. Allen Cowley, Jr. described his ongoing interest in balancing reductionist and genomic-scale analytical approaches linking physiology to the genome. The closing keynote lecture featured distinguished National Academy of Science member Dr. Aravinda Chakravarti, a prominent human geneticist who proposed tantalizing views of the heritability of complex disease and his emphasis on gene regulatory networks in phenotypes.
Conference organizers were challenged to address the future of the Rat Genomics and Models meeting. It was overwhelmingly agreed by both investigators and trainees that this meeting serves a pivotal role by bringing together a diverse set of researchers who deploy the laboratory rat model across a range of biological, physiological, and behavioral genomic research. The wide range of fundamental research areas and technologies presented distinguishes this meeting from others. Several attendees opined that the insight into cutting-edge scientific information shared at this meeting continues to be of tremendous value toward defining the scientific strategies in their own laboratories. New possibilities were identified to enhance the meeting’s vitality and attract new investigators and trainees, including the organization of specialized workshops and satellite events. The organizers and attendees are devoted to improving, growing, and continuing the present biannual meeting format at the Cold Spring Harbor Laboratory and other sites in the intervening years.

PROGRAM

Keynote Address
A. Cowley, Jr., Medical College of Wisconsin, Milwaukee

Metabolism, Inflammation, Exercise Physiology
Chairperson: A. Geurts, Medical College of Wisconsin, Milwaukee

Manipulating the Genome
Chairperson: A. Geurts, Medical College of Wisconsin, Milwaukee

Integrative Biology and Genomics
Chairperson: E. Petretto, Imperial College London, United Kingdom

Emerging Areas
Chairperson: M. Gould, University of Wisconsin, Madison

Tumor Biology
Chairperson: M. Gould, University of Wisconsin, Madison

Cardiovascular
Chairperson: B. Joe, University of Toledo, Ohio

Rat Resources
Chairperson: A. Geurts, Medical College of Wisconsin, Milwaukee

Keynote Address
A. Chakravarti, Johns Hopkins University, Baltimore, Maryland

Neurobiology and Addiction
Chairperson: B. Tabakoff, University of Colorado Anschutz Medical Campus, Aurora

A. Takizawa, R. Schilling
D. Rubinstein, K. Krentz, J. Mullins
A. Kindler Röhrborn, F. Albert
POSTGRADUATE COURSES

Workshop on Leadership in Bioscience

March 13–16

INSTRUCTORS
C. Cohen, Science Management Associates, Newtown, Massachusetts
D. Kennedy, WorkLab Consulting, New York

This intensive and interactive 4-day workshop has been held each winter since 2011. Previously funded by American Express Philanthropy, it is now funded by a new National Institutes of Health NIGMS grant for Innovative Programs to Enhance Research Training. The primary goal of the workshop is to develop skills necessary for leading and interacting effectively with others in both one-on-one and group settings, particularly independent laboratory settings in academic institutions. Modules include Case Studies, Fundamentals of Negotiation, Group Dynamics and Meetings, Projecting Leadership, and Feedback and Evaluation for Scientists. The 2015 workshop trained 22 students, primarily postdoctoral fellows and junior faculty from all areas of biology. CSHL will continue to offer the leadership workshop each winter or spring through at least 2019.

This course was supported with funds provided by the National Institute of General Medical Sciences.

PARTICIPANTS
Anthony, T., Ph.D., California Institute of Technology, Pasadena
Ballouz, S., Ph.D., Cold Spring Harbor Laboratory
Berger, A., Ph.D., Broad Institute, Cambridge, Massachusetts
Casteel, C., B.S., University of California, Davis
Clugston, R., M.Sc., Columbia University, New York
Cotney, J., Ph.D., University of Connecticut School of Medicine, Farmington
Esko, T., M.Sc., Boston Children’s Hospital, Boston, Massachusetts
Fratet-Turcotte, A., B.Sc., Mount Sinai Hospital, Toronto, Canada
Hsu, C., B.Sc., University of Calgary, Canada
Hsu, Y.-C., B.S., Harvard University, Cambridge, Massachusetts
Karlstrom, H., B.Sc., Karolinska Institutet, Stockholm, Sweden
Kim, Y., B.S., Cold Spring Harbor Laboratory
Kumar, V., Ph.D., The Jackson Laboratory, Bar Harbor, Maine

Michener, J., Ph.D., Massachusetts Institute of Technology, Cambridge
Mills, K., B.S., Rensselaer Polytechnic Institute, Troy, New York
Mustapha, M., B.S., M.S., Stanford University, Stanford, California
Neunuebel, M., B.A., University of Delaware, Newark
Orthwein, A., B.Sc., Mount Sinai Hospital, Toronto, Canada
Pajvani, U., B.S., Columbia University, New York
Pntziachristos, P., B.Sc., New York University Langone Medical Center, New York
Ralston, K., B.S., University of California, Davis, California
Schoenfeld, E., B.A., Massachusetts Institute of Technology, Cambridge
Protein Purification and Characterization

April 8–21

INSTRUCTORS
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
J. Love, Albert Einstein College of Medicine, Bronx, New York
S. Nechaev, University of North Dakota School of Medicine, Grand Forks

ASSISTANTS
A. Abbas, University of North Dakota School of Medicine, Grand Forks
M. Chambers, University of California, Los Angeles
N. Clark, Brandeis University, Waltham, Massachusetts
M. Donovan, Brandeis University, Waltham, Massachusetts
M. Kim, University of California, Los Angeles
Y.-C. Lee, MD Anderson Cancer Center, Houston, Texas
A. Samarakkody, University of North Dakota School of Medicine, Grand Forks
A. Sawyer, Brandeis University, Waltham, Massachusetts
M. Spellberg, Brandeis University, Wellesley, Massachusetts

This course was organized for scientists who are not familiar with the techniques of protein isolation and characterization. The course was extremely rigorous and included laboratory work during the day, as well as lectures with discussions and student talks in the evenings. Students were 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.

The students became familiar with major techniques in protein purification by performing multiple protein isolations including (1) a regulatory protein (calmodulin) from brain tissue, (2) a
sequence-specific DNA-binding protein (transcription factor AP1) as well as RNA polymerase II from HeLa cell nuclei, (3) recombinant proteins overexpressed as inclusion bodies in *Escherichia coli* and tagged with a variety of different affinity tags, including His tags, GST, SBP, MBP, etc., and (4) membrane-bound proteins, including insulin receptor from rat liver and recombinant human integral membrane proteins from HEK293 cells.

Participants were divided into groups and spent 3 days in each of four modules. A number of relevant characterizations were performed on each protein, providing students experience with immunological and biochemical assays, peptide mapping, protein sequencing, and mass spectrometry. Students learned bulk fractionation, 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. They also learned procedures for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. For membrane proteins, students learned how to deal with low-expressing proteins, detergent selection, and detergent concentration assays. 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

Aubee, J., B.S., Howard University College of Medicine, Washington, D.C.
Cassina, V., Ph.D., Università degli Studi di Milano-Bicocca, Monza, Italy
Garcia, V., Ph.D., Carnegie Science Institution for Science, Stanford, California
Gebbie, M., B.S., University of Maryland, College Park
Karlsson, E., M.S., Chalmers University of Technology, Gothenburg, Sweden
Hughes, L.L., Ph.D., Instituto de Investigaciones Biológicas Clemente Estable, Montevideo, Uruguay
Modica, C., B.A., University of Buffalo, New York
Patel, S., M.S., Memorial Sloan Kettering Cancer Center, New York
Sarowar, S., M.S., Brookhaven National Laboratory, Rocky Point, New York
Skipwith, C., Ph.D., Northeastern University, Boston, Massachusetts
Song, D., M.S., Cold Spring Harbor Laboratory
Wang, J.X., M.Sc., Cold Spring Harbor Laboratory

SEMINARS

Courey, A., University of California, Los Angeles: Welcome and Introduction to Course. System-wide analyses of Groucho and SUMO in *Drosophila*.
Cowburn, D., Albert Einstein School of Medicine, Bronx, New York: Protein engineering using inteins.
Love, J., Albert Einstein School of Medicine, New York: High-throughput production of eukaryotic soluble and membrane proteins.
Marr, M., Brandeis University, Boston, Massachusetts: Introduction to protein purification. Controlling gene expression in response to stress.
Nechaev, S., University of North Dakota, Grand Forks: Stable pol II pausing is retained during gene activation to provide a platform for regulation.
Lin, S.-H., MD Anderson Center Center/University of Texas, Houston: Secreted factors in the tumor microenvironment confer therapy resistance.
Quantitative Imaging: From Cells to Molecules

April 8–21

INSTRUCTORS

H. Elliott, Harvard Medical School, Boston, Massachusetts
J. Waters, Harvard Medical School, Boston, Massachusetts
T. Wittmann, University of California, San Francisco

CO-INSTRUCTOR

T. Lambert, Harvard Medical School, Boston, Massachusetts

ASSISTANTS

A. Ettinger, University of California, San Francisco
A. Jost, Harvard Medical School, Boston, Massachusetts
P. Pitrone, MPI-CBG, Dresden, Saxony, Germany
P. Tomancak, MPI-CBG, Dresden, Saxony, Germany
J. van Haren, 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. It was designed for cell and molecular biologists with little or no microscopy experience who wish to begin utilizing microscopy in their own research. Students gained a theoretical understanding of, and hands-on experience with, state-of-the-art equipment used in quantitative fluorescence microscopy including wide-field fluorescence microscopy, laser-scanning and spinning-disk confocal microscopy, total internal fluorescence microscopy (TIRF), super-resolution methods (structured illumination, STED, 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 quantitative microscopy basics, transmitted light microscopy, image segmentation, image analysis, CCD and sCMOS cameras, confocal microscopy, multiphoton microscopy, deconvolution, TIRF, imaging ratiometric “biosensors” (including FRET), light sheet microscopy, and super-resolution techniques. Students also learned guidelines for choosing fluorescent proteins and worked with live samples requiring environmental control. In addition to the course instructors listed above, lecturers included Nathan Shaner (the Scintillon Institute), Talley Lambert (Harvard Medical School), Dylan Burnette (Vanderbilt), Julie Canman (Columbia University), Scott Fraser (University of Southern California), Rebecca Heald (University of California, Berkeley), Elizabeth Hillman (Columbia University), Pavel Tomancak (Max-Planck Institute of Molecular Cell Biology & Genetics), and Jeffrey van Haren (University of California).

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Azoitei, M., Ph.D., University of North Carolina at Chapel Hill
Bousbaine, D., B.S., M.S., Massachusetts Institute of Technology, Cambridge
Burke, R., B.S., University of Colorado, Boulder
Cheung, L., Ph.D., Carnegie Institution for Science, Stanford, California
Chow, T., Ph.D., University of California, San Francisco
Kulkami, S., Ph.D., Yale School of Medicine, New Haven, Connecticut
Lee, J.-Y., Ph.D., University of California, Berkeley
McQuate, S., Ph.D., University of Colorado, Boulder
Moison, E., B.S., M.S., Harvard University, Cambridge, Massachusetts
Salogiannis, J., Ph.D., Harvard Medical School, Boston, Massachusetts
Stein, V., M.S., University of Queensland, Brisbane, Australia
Sundaramoorthy, S., Ph.D., Columbia University Medical Center, New York
Taveras, C., B.S., Columbia University, New York
Wolff, S., Ph.D., University of North Carolina, Chapel Hill
Yuan, S., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Zheng, Y., Ph.D., Weill Cornell Medical College, New York

SEMINARS

Ettinger, A., University of California, San Francisco: To curve or not curve: Doublecortin-microtubule interactions.
Burnette, D., Vanderbilt School of Medicine, Brentwood, Tennessee: Super-resolution microscopy. Studying cellular contractility with super-resolution microscopy.
Hillman, E., Columbia University, New York: Swept, confocally aligned planar excitation (SCAPE) microscopy for fast in vivo volumetric imaging.
van Haren, J., University of California, San Francisco: Light-control of microtubule dynamics.

Moison, E., B.S., M.S., Harvard University, Cambridge, Massachusetts
Salogiannis, J., Ph.D., Harvard Medical School, Boston, Massachusetts
Stein, V., M.S., University of Queensland, Brisbane, Australia
Sundaramoorthy, S., Ph.D., Columbia University Medical Center, New York
Taveras, C., B.S., Columbia University, New York
Wolff, S., Ph.D., University of North Carolina, Chapel Hill
Yuan, S., Ph.D., Yale University School of Medicine, New Haven, Connecticut
Zheng, Y., Ph.D., Weill Cornell Medical College, New York

Canman, J., Columbia University, New York: The molecular choreography of cell division.
Shaner, N., The Scintillon Institute, San Diego, California: Fluorescent proteins.
Heald, R., University of California, Berkeley: Mechanisms of mitosis and size control in Xenopus.
Fraser, S., University of Southern California, Los Angeles: Sensing and imaging the molecular signature of embodied cells.
Wittmann, T., University of California, San Francisco: Live confocal microscopy, FRAP, and photo-activation. TIRF.
Cell and Developmental Biology of *Xenopus*

April 9–21

INSTRUCTORS
K. Liu, Kings College, London, United Kingdom
M. Khokha, Yale University, New Haven, Connecticut

ASSISTANTS
J. Oomen-Hajagos, Stony Brook University, Stony Brook, New York
E. Mis, Yale University School of Medicine, New Haven, Connecticut
B. Steventon, University of Southern California, Los Angeles

*Xenopus* is an extraordinary in vivo model for cell and developmental biology. The ease of loss- and gain-of-function approaches allows rapid mechanistic analysis; this can be combined with classic embryological manipulations and state-of-the-art imaging. As advances in human genomics rapidly expand our list of novel disease genes, *Xenopus* is emerging as a powerful, high-throughput system for studying unknown gene function. Techniques covered in this course included microinjection, molecular manipulations such as CRISPR knockouts, transgenics, and mRNA overexpression. These were combined with explant and transplant methods to simplify or test tissue-level interactions. To visualize subcellular and intercellular activities, we introduced a variety of imaging methods including time-lapse, fluorescent, and confocal microscopy. Additional methods included mRNA in situ hybridization and protein immunohistochemistry as well as basic bioinformatics techniques for gene comparison and functional analysis. This course was designed for those new to the *Xenopus* field, as well as for more advanced students who were interested in emerging technologies. We encouraged students to bring their own genes of interest and we tailored aspects of the course to enable them to initiate studies on their specific projects.
This course was supported with funds provided by the National Institute of Child Health and Human Development and the Howard Hughes Medical Institute.

PARTICIPANTS

Angerilli, A., B.A., M.S., Ludwig Maximilian Universitat, Muenchen, Germany
Banach, M., B.A., University of Rochester Medical Center, New York
Cunha, D., B.Ch., Federal University of Rio de Janeiro, Brazil
DeLay, B., Ph.D., University of Texas Medical School, Houston
Esmaeili, M., B.A., University of Pennsylvania School of Medicine, Philadelphia
Guo, S., Ph.D., Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio
Huebner, R., B.S., Johns Hopkins University School of Medicine, Baltimore, Maryland
Kha, C., B.S., University of Las Vegas, Nevada
Lopez Munoz, A., B.Sc., King’s College London, United Kingdom
Miller, K., B.S., University of California, Berkeley
Owens, N., Ph.D., MRC National Institute for Medical Research, London, United Kingdom
Peuchen, E., B.S., University of Notre Dame, Notre Dame, Indiana
Rankin, H., B.S., Yale University, New Haven, Connecticut
Toro-Tapia, G., M.S., Universidad de Concepción, Chile
Zapata, J., Ph.D., MD Anderson Cancer Center, Houston, Texas

SEMINARS

El-Hodiri, H., Nationwide Children’s Hospital Research Institute, Columbus, Ohio: The retinal homeobox (Rx) gene and retinal progenitor cells.
Cousin, H., University of Massachusetts, Amherst: Xenopus: The perfect model system to study early events of craniofacial development.
Thomsen, J., Stony Brook University, Stony Brook, New York: Xenopus show and tell.
Wallingford, J., University of Texas, Austin: Adventures in the ignorome: Developmental systems biology and search for novel protein functions.
Davidson, L., University of Pittsburgh, Pennsylvania: Mechanical feedback during morphogenesis and self-assembly.
Kirschner, M., Harvard Medical School, Boston, Massachusetts: Dynamics of protein and RNA expression in developmental systems.

Papalopulu, N., University of Manchester, United Kingdom: From Xenopus and beyond: How the dynamics of gene expression changed our understanding of cell state transitions.
Miller, R., University of Texas Medical School, Houston: Wnt you like to know about kidney tubulogenesis and cilia?
Keller, R., University of Virginia, Charlottesville: The integrated machines of Xenopus.
Heald, R., University of California, Berkeley: Mechanisms of mitosis and size control in Xenopus.
Fraser, S., University of Southern California, Los Angeles: Sensing and imaging the molecular signatures of embodied cells.
The goal of this 2-week course was to familiarize students with the most recent cutting-edge technologies for characterization of single 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 expert in particular areas of single-cell analysis. Topics covered included quantitative single-cell analysis by RNA-Seq, genomic DNA analysis, epigenetics, RNA localization analysis, proteomics, protein translation, and metabolomics. The techniques taught included real-time live-cell quantification where appropriate. Multiple nucleic
amplification methodologies including aRNA, single-cell PCR, MALBAC, and MDA were used. Further multiple techniques to isolate specific cell populations and individual cells were also used. The course included the use of model systems such as mouse, rat, Aplysia, and *Caenorhabditis elegans*.

This course was supported with funds provided by the National Institute of General Medical Sciences, Helmsley Charitable Trust, and the Howard Hughes Medical Institute.

**PARTICIPANTS**

Cantone, I., Ph.D., MRC Clinical Sciences Centre, London, United Kingdom
Clark, I., Ph.D., University of California, San Francisco
Coppola, G., Ph.D., Yale School of Medicine, New Haven, Connecticut
de Caceres-Bustos, A., Ph.D., Duke University, Durham, North Carolina
Durante, M., B.Sc., University of Miami, Florida
Ellwanger, D., M.Sc., Technical University of Munich, Freising, Germany
Haug, J., B.S., Stowers Institute, Kansas City, Missouri
Lee, B.-M., D.D.S., Seoul National University, Seoul, South Korea
Masserdotti, G., Ph.D., Helmholtz-Zentrum, Neuherberg, Germany
Mathur, C., Ph.D., NINDS/NIH, Bethesda, Maryland
McGovern, A., Ph.D., The University of Queensland, St. Lucia, Australia
Mendivil Ramos, O., Ph.D., Cold Spring Harbor Laboratory
Patel, S., B.Phar., Memorial Sloan Kettering Cancer Center, New York
Seib, P., Ph.D., University of Strathclyde, Glasgow, United Kingdom
Shi, Y., M.D., University of Virginia, Charlottesville
Singh, S., Ph.D., Mount Sinai School of Medicine, New York
Wilson, R., B.S., Stanford University, California

**SEMINARS**

Herr, A., University of Berkeley, California: Microfluidic tools design for protein profiling of single cells.
Nolan, G., Stanford University School of Medicine, California: The deeper phenotypes of the single cell in cancer and immunity.
Sweedler, J., University of Illinois, Urbana: Mass spectrometry-based approaches to enable the cell-by-cell chemical characterization of the brain.
Baldwin, K., The Scripps Research Institute, La Jolla, California: Reprogramming and lineage-based approach to single-cell whole-genome analyses of somatic mutation.
McConnell, M., University of Virginia, Charlottesville: Single-cell genome sequencing reveals mosaic CNVs in human neurons.
Albritton, N., University of North Carolina, Chapel Hill: Microengineered tools for single cells.
Fan, R., Yale University, New Haven, Connecticut: Single-cell, 42-plex cytokine analysis of immune defense and hematopoietic malignancy.
Advanced Bacterial Genetics

June 3–23

INSTRUCTORS
D. Hughes, Uppsala University, Sweden
B. Lazazzera, University of California, Los Angeles
F. Yildiz, University of California, Santa Cruz

ASSISTANTS
S. Elgamal, The Ohio State University, Columbus
N. (Jiunn) Fong, University of California, Santa Cruz
E. Garmendia, Uppsala University, Sweden

This course presented logic and methods used in the genetic dissection of complex biological processes in diverse bacteria. Laboratory methods included classical mutagenesis using transposons, mutator strains, and chemical mutagens; recombineering with single- and double-stranded DNA; detection of gene expression changes using various reporter genes; the mapping of mutations using genetic and physical techniques; modern approaches to the generation and analysis of targeted gene disruptions and fusions using 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 Escherichia coli, Salmonella, Bacillus subtilis, and Vibrio cholerae), and the use of the wealth of new genomic sequence information to motivate these methods. Invited lecturers presented various genetic approaches to study bacterial mechanisms of metabolism, development, and pathogenesis. The course admitted 16 students, both foreign and U.S. residents, from diverse backgrounds and career levels for intensive (but fun) instruction in microbial genetics.

This course was supported with funds provided by the National Science Foundation and Helmsley Charitable Trust.
PARTICIPANTS

Allemann, M., B.S., Scripps Institution of Oceanography, University of California, San Diego, La Jolla, California
Andrade, M., Ph.D., Citrus Research and Education Center/University of Florida, Lake Alfred
Bigosch, C., M.S., Eawag, Dübendorf, Switzerland
Brown, S., Ph.D., University of California, San Diego
Cambre, A., M.S., KU Leuven, Heverlee, Belgium
Chu, K.-H., Ph.D., Texas A&M University, College Station
Cohen, E., B.S., University of Utah, Salt Lake City
Esquivel, S., B.A., University of Utah, Salt Lake City
Hindes, D., B.S., University of Albany, New York
Hu, L., Ph.D., University of Wisconsin, Madison
Huang, G., B.S., University of California, Los Angeles
Igler, C., M.S., Institute of Science and Technology, Klosterneburg, Austria
Manjarrez Casas, A., M.Sc., ETH Zurich & Eawag, Dübendorf, Switzerland
Nuester, E., Ph.D., California State University, Chico
Vestö, K., B.S., Karolinska Institutet, Stockholm, Sweden
Wollrab, E., Ph.D., Institut Pasteur, Paris, France

SEMINARS

Camilli, A., Tufts University Medical School, Boston, Massachusetts: Transposon-sequencing (Tri-Seq) to find important genes of pathogens.
Andersson, D., Uppsala University, Sweden: Evolution of antibiotic resistance at low antibiotic levels.
Low, D., University of California, Los Angeles: Interbacterial communication, a touching story.
Miller, J., University of California, Los Angeles: Diversity-generating retroelements in bacteria, archaea, and their viruses.
Ruby, E., University of Wisconsin, Madison: Tales from the crypts: Gene expression at the single-cell level in the squid–Vibrio symbiosis.
Bossi, L., Université Paris-Sud, Paris, France: Multiple facets of Rho-mediated transcription termination.
Marraffini, L., The Rockefeller University, New York: Molecular mechanisms of CRISPR–Cas immunity.
Taylor, R., Geisel School of Medicine at Dartmouth, Hanover, New Hampshire: Proteolysis of virulence regulator ToxR is associated with entry of Vibrio cholera into a dormant state.
Sperandio, V., University of Texas Southwestern Medical School, Dallas: Inter-kingdom signaling in host–microbiota–pathogen interactions.
Ion Channels and Synaptic Transmission

June 3–23

INSTRUCTORS
T. Branco, MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
S. Brenowitz, HHMI/Janelia Farm Research Campus, Auburn, Virginia
I. Duguid, University of Edinburgh, United Kingdom
P. Kammermeier, University of Rochester Medical Center, New York

ASSISTANTS
J. Dacre, University of Edinburgh, United Kingdom
J. Lueck, University of Iowa, Iowa City
A. Matsui, National Institutes of Health, Rockville, Maryland
M. Scott, University of Rochester, New York
N. Wanaverbecq, Aix-Marseille University, France

The primary goal of this course was to investigate, through lectures and laboratory work, the properties of ion channels that allowed neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitter at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) 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 and neurons in brain slice preparations and in the mouse brain in vivo. 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 with respect 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 with funds provided by Helmsley Charitable Trust and the Howard Hughes Medical Institute.

PARTICIPANTS

Antoine, M., Ph.D., University of California, Berkeley
Benthall, K., Ph.D., University of California, Berkeley
Beskow, A., Ph.D., Columbia University, New York
Bissen, D., M.S., Max-Planck Institute of Brain Research, Frankfurt am Main, Germany
Callan, A., B.S., Ludwig Maximilian University of Munich, Planegg-Martinsried, Germany
Caval-Holme, F., B.S., University of California, Berkeley
Dolan, M.-J., B.Sc., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom
Kim, E., Ph.D., Northeastern University, Boston, Massachusetts
Liu, J., M.S., California Institute of Technology, Pasadena
Puhl, C., B.Sc., King's College London, United Kingdom
Valakh, V., B.S., Washington University, St. Louis, Missouri
Zheng, Y., B.S., Harvard Medical School, Boston, Massachusetts

SEMINARS

Lee, A., University of Iowa, Iowa City: Ca channels.
Plested, A., Leibnitz Institute for Molecular Pharmacology, Berlin, Germany: Glutamate receptors.
Clark, B., University College London, United Kingdom: Na channels.
Montell, C., University of California, Santa Barbara: TRP channels.
Nimigean, C., Weill Cornell Medical College, New York: Voltage-gated channels/K⁺.
Magee, J., HHMI/Janelia Farm Research Campus, Ashburn, Virginia: Final lecture.
Sjostrom, J., McGill University, Montreal, Quebec, Canada: Spike-timing-dependent plasticity in neocortex.
Dudman, J., Janelia Farm Research Campus, Ashburn, Virginia: In vivo recording.
Shah, M., University College London, United Kingdom: HCN channels.
Mouse Development, Stem Cells, and Cancer

June 3–23

INSTRUCTORS  M. Lewandoski, National Cancer Institute, Frederick, Maryland  
D. Wellik, University of Michigan Medical Center, Ann Arbor

CO-INSTRUCTORS  A. Ralston, Michigan State University, E. Lansing  
L. Selleri, Weill Cornell Medical College, New York

ASSISTANTS  C. Cebrian, University of Michigan, Ann Arbor  
T. Frum, Michigan State University, E. Lansing  
A. Lokken, Michigan State University, E. Lansing  
M. Newman, University of Michigan, Ypsilanti  
K. Pinaeult, University of Michigan, Ann Arbor  
D. Rux, University of Michigan, Ann Arbor

This intensive lecture and laboratory course was designed for scientists interested in using mouse models to study mammalian development, stem cells, and cancer. The lecture portion of the course, taught by leaders in the field, provided the conceptual basis for contemporary research in embryogenesis, mathematical modeling of disease and development, organogenesis, embryonic, adult and induced pluripotent stem cells, and cancer biology.

The laboratory and workshop portions of the course provided a hands-on introduction to engineering of mouse models, stem-cell technologies, and tissue analyses. Experimental techniques included genome editing by CRISPR/Cas9, pronuclear microinjection, isolation and
culture/manipulation of pre- and postimplantation embryos, embryo transfer, embryo electroporation and roller bottle culture, chimera generation, generation and differentiation of mouse embryonic stem cells, as well as induced pluripotent stem cells, isolation of mouse embryonic fibroblasts, vibratome and cryosectioning, in situ RNA hybridization, immunostaining, FACS sorting and analysis, vascular injections and casting, neural stereotaxic injections, skeletal preparation, organ explant culture and fluorescent imaging, including live time-lapse microscopy and tumors.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Asani, M., M.S., SUNY Downstate Medical Center, Brooklyn, New York
Biswas, S., M.S., University of Kentucky, Lexington
Chi, F., B.S., University of California, Los Angeles
Guha, A., Ph.D., Boston University Medical Campus, Boston, Massachusetts
Li, C., B.S., Cincinnati Children's Hospital Medical Center, Ohio
Libreros, S., B.S., Florida Atlantic University, Boca Raton
Liu, H., Ph.D., Regenerative Bioscience Center, Athens, California
Lungova, V., Ph.D., University of Wisconsin, Madison
Mlynarczyk, C., Ph.D., Weill Cornell Medical College, New York
Raspopovic, J., Ph.D., Friedrich Miescher Laboratory of the MPI, Tübingen, Germany
Rozhkov, N., Ph.D., Cold Spring Harbor Laboratory
Tuck, A., Ph.D., Friedrich Miescher Institute, Basel, Switzerland
Van Treuren, T., B.S., University of North Texas Health Science Center, Fort Worth
Xie, Y., B.S., University of Utah, Salt Lake City

SEMINARS

Ralston, A., University of California, Santa Cruz: Stem cell origins in the mouse blastocyst.
Arenkiel, B., Baylor College of Medicine, Houston, Texas: Conditional viral genetic approaches to study the nervous system.
Cebrian, C., University of Michigan, Ann Arbor: MADM and live imaging to study single-cell behavior.
Threadgill, D., North Carolina State University, Raleigh: Historical overview of the mouse as a model system.
Quantitative genetics applied to development.
Solter, D., Max-Planck Society, Bar Harbor, Maine: Preimplantation development.
Wellik, D., University of Michigan, Ann Arbor: Hox genes in development and repair.
Wang, H., The Jackson Laboratory, Bar Harbor, Maine: Genome editing in stem cells and mice. Cas/CRISSPR Workshop.
Varmus, H., Weill Cornell Medical College, New York: Mouse models and cancer.
Maillard, I., University of Michigan, Ann Arbor: Hematopoiesis.
Glazer, J., Indiana University, Bloomington: Virtual-tissue computer simulations to explore developmental mechanisms.
Sanes, J., Harvard University, Cambridge, Massachusetts: Using genetic methods in mice to understand the nervous system.
Hadjantonakis, K., Memorial Sloan Kettering Cancer Center, New York: Optical imaging of mouse embryos.
Capecchi, M., University of Utah School of Medicine/HHMI, Salt Lake City: Mouse models of human disease from cancer to neuropsychiatric disorders.
Lewandoski, M., National Cancer Institute, Frederick, Maryland: Somitogenesis. Technology (minus MADAM).
Egeblad, M., Cold Spring Harbor Laboratory: Intravital imaging in living mice; visualizing drug resistance and metastasis.
Klein, O., University of California, San Francisco: Mammalian epithelial stem cells: An overview.
P Tam, P., Children's Medical Research Institute, Australia: Gastrulation: Lineage allocation and embryonic patterning (4th to 9th).
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: Neural crest cells: Their roles in evolution, development, disease, and cancer.
Lowe, S., Memorial Sloan Kettering Cancer Center, New York: Probing cancer drivers and dependencies using mouse models and RNAi.
Huppert, S., Cincinnati Children's Hospital Medical Center, Ohio: Liver development and regeneration: Regulating cell lineage decisions.
Yamaguchi, T., National Cancer Institute, Frederick, Maryland: Trunk progenitors and axis extension.
Sun, X., University of Wisconsin, Madison: Dissecting lung development and disease using mouse models.
Workshop on Autism Spectrum Disorders

June 4–10

INSTRUCTORS  D. Geschwind, University of California Center for Autism Research
               S. Spence, Children’s Hospital, Boston

CO-INSTRUCTORS  G. Fishell, New York University, New York
                 J. McPartland, Yale University, New Haven, Connecticut
                 J. Veenstra-Vanderweele, Columbia University, New York

ASSISTANT  M. Lazaro, University of California, Los Angeles

Complex diseases are conditions that are influenced by the actions of multiple genes and their interactions with each other and with the environment. This lecture course considered the major strategies for studying the genetic basis of complex disorders such as diabetes, cardiovascular disease, cancer, Alzheimer’s disease, schizophrenia, and epilepsy. We discussed genetic-epidemiologic study designs and methods for quantifying the strength of the genetic influences on a disease. A major focus was the analysis of exome chip, exome sequencing data, and whole-genome sequencing data. Also discussed were the efficiency and robustness of different designs for such analysis and how evidence from epidemiologic studies informs both the design and interpretation of molecular genetic studies. Study design and methods for analysis of quantitative risk factors related to complex diseases were covered as well as haplotype mapping, analysis of structural variation, meta-analysis, and gene–environment interaction. An overview of high-throughput laboratory methods was given to provide participants with insight into the applications of these techniques.
Illustrations were provided through discussion of results from ongoing studies of a variety of complex diseases and related risk factors.

This course was supported with funds provided by the Nancy Lurie Marks Family Foundation.

PARTICIPANTS

Avillach, P., Ph.D., Harvard Medical School, Boston, Massachusetts
Chenausky, K., Ph.D., Boston University, Massachusetts
Choe, K., Ph.D., University of California, Los Angeles
Crippa, A., M.S., Scientific Institute, Bosisio Parini, Italy
Fiella, M., Ph.D., Vanderbilt University, Nashville, Texas
Folkes, O., Ph.D., Vanderbilt University, Nashville, Texas
Gao, X., B.S., Massachusetts Institute of Technology, Cambridge
Ghosh, M., Ph.D., University College London, United Kingdom
Hagashimori, H., Ph.D., Tufts University, Medford, Massachusetts
He, C., M.D./Ph.D., UCLA, Los Angeles, California
Jouravlev, O., Ph.D., Massachusetts Institute of Technology, Cambridge
Kazlauskas, N., Ph.D., IFIByNE-CONICET, Buenos Aires, Argentina
Kislin, M., Ph.D., University of Helsinki, Finland
Kozol, R., Ph.D., University of Miami, Coral Gables, Florida
Mody, M., Ph.D., Lurie Center for Autism, Lexington, Massachusetts
Muhle, R., M.D./Ph.D., Yale University, New Haven, Connecticut
Pinto, B., Ph.D., Italian Institute of Technology, Genoa, Italy
Rehg, J., Ph.D., Georgia Institute of Technology, Atlanta
Scharf, S., Ph.D., F. Hoffmann–La Roche AG, Basel, Switzerland
Steele, K., M.S., Albert Einstein College of Medicine, Bronx, New York
Tao, J., Ph.D., Massachusetts Institute of Technology, Cambridge
Tylee, D., M.D./Ph.D., SUNY Upstate Medical, Syracuse, New York

SEMINARS

Geschwind, D., University of California, Los Angeles, and Spence, S., Boston Children’s Hospital, Massachusetts: Course overview. Introduction to human genetics and genomics. Genetic findings in autism spectrum disorders. Basic of neural development and neuropathology.
Isabelle Rapin, I., Albert Einstein School of Medicine, Bronx, New York: Autism’s odyssey: 1943–2015.
Veenastra-Vanderweele, J., Columbia University, New York: Clinical presentation and phenotypic variation. From mouse to man: The path to new treatment in autism spectrum disorders.
Spence, S., Boston Children’s Hospital, Massachusetts: Medical co-morbidities.
Fombonne, E., Oregon Health and Science University, Portland: Epidemiology of autism spectrum disorders.
Weisskopf, M., Harvard School of Public Health, Boston, Massachusetts: The epidemiology of environmental toxicants and autism spectrum disorders.

Mill, J., University of Exeter Medical School, United Kingdom: Epigenetics in autism spectrum disorders.
Hench, T., Harvard University, Boston, Massachusetts: Critical periods/Plasticity mechanisms.
Fishell, G., New York University, New York: Synaptic physiology/E-I balance/role of interneurons.
Kasari, C., University of California, Los Angeles: Current treatments overview.
Fagiolini, M., Boston Children’s Hospital, Massachusetts: Animal models of autism spectrum disorders.
Feng, G., Massachusetts Institute of Technology, Cambridge: Neural circuit dissection/contribution of basal ganglia to behavior and dysfunction in animal models.
Amaral, D., MIND Institute, Sacramento, California: Neuroanatomy of autism spectrum disorders.
Murphy, D., Kings College London, United Kingdom: Neuroimaging findings in autism spectrum disorders.
Happe, F., Institute of Physics, London, United Kingdom: Psychological models of autism spectrum disorders.
Rogers, J., Southwest Foundation for Biomedical Research, San Antonio, Texas: Evolutionary perspective on social behavior.
Iversen, P., Cure Autism Now: Perspectives in autism.
Statistical Methods for Functional Genomics

June 18–July 1

INSTRUCTORS  
H. Bussemaker, Columbia University, New York  
S. Davis, National Institutes of Health, Bethesda, Maryland  
O. Elemento, Weill Cornell Medical College, New York  
R. Irizarry, Harvard/Dana-Farber Cancer Institute, Brookline, Massachusetts

ASSISTANTS  
V. FitzPatrick, Columbia University, New York  
S. Hicks, Dana-Farber Cancer Institute, Boston, Massachusetts  
G. Martini, Columbia University, New York  
C. Rastogi, Columbia University, New York

Over the past decade, high-throughput assays have become pervasive in biological research because of 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 is 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 the National Institute of General Medical Sciences.
PARTICIPANTS

Abdullayev, I., M.S., Karolinska Institutet, Stockholm, Sweden
Barisic, D., M.Sc., Friedrich Miescher Institute, Basel, Switzerland
Chapman, L., B.S., University of Rochester School of Medicine, West Henrietta, New York
Ding, Y., Ph.D., Regeneron Pharmaceuticals, Tarrytown, New York
Gallego, J., M.D., The Zucker Hillside Hospital, Glen Oaks, New York
Gardiner, D., Ph.D., CSIRO, St. Lucia, Australia
Hannon, C., B.S., Princeton University, New Jersey
Henry, F., Ph.D., HHMI/Janelia Research Campus, Ashburn, Virginia
Kinde, B., B.S., Harvard Medical School, Boston, Massachusetts
King, B.S., Ph.D., Washington University, St. Louis, Missouri
LaRue, R., Ph.D., University of Minnesota, Minneapolis
Lozano, R., Ph.D., Cornell University, Ithaca, New York
Malhotra, D., Ph.D., F. Hoffman La-Roche Ltd., Basel, Switzerland
Prokop, J., Ph.D., Medical College of Wisconsin, Milwaukee
Pucella, J., Ph.D., Memorial Sloan Kettering Cancer Center, New York
Ramos, P., Ph.D., Medical University of South Carolina, Charleston
Schwaiger, M., Ph.D., Novartis Institutes for BioMedical Research, Basel, Switzerland
Shah, N., B.S., Memorial Sloan Kettering Cancer Center, New York
Sheynkman, G., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts
Singh, P., Ph.D., University of Buffalo, New York
Teich, A., Ph.D., Columbia University, New York
Tsai, H.-C., Ph.D., National Taiwan University, Taipei
Tsompana, M., Ph.D., SUNY at University of Buffalo, New York
Ziegler, K., Ph.D., University of Alberta, Edmonton, Canada

SEMINARS

Kundaje, A., Stanford University, California: Introduction to ENCODE and ChiP-Seq.
Carlson, M., Fred Hutchinson Cancer Research Center, Seattle, Washington: Introduction to bioconductor annotation resources.

Davis, S., National Institutes of Health, Bethesda, Maryland: High-resolution views of the genome. Exploratory data analysis for RNA-Seq.
Satija, R., New York Genome Center/New York University, New York: Learning the metadata of the cell, statistical methods for single-cell genomics.
Tuuli Lappalainen, T., New York Genome Center/Columbia University, New York: Integrating transcriptome and genome sequencing to understand functional variation in human genomes.
Workshop on Pancreatic Cancer

June 24–30

INSTRUCTORS

D. Bar-Sagi, New York University Langone Medical Center, New York
S. Leach, Memorial Sloan Kettering Cancer Center, New York
A. Maitra, MD Anderson Cancer Center, Houston, Texas
D. Tuveson, Cold Spring Harbor Laboratory

Pancreatic cancer is one of the deadliest cancers. Tumors are often diagnosed at advanced stages of the disease and metastasize rapidly. This 1-week discussion course provided a comprehensive overview of clinical and biological aspects of pancreatic cancer with special emphasis on disease diagnosis and management, molecular pathways involved in tumor development and progression, mechanism-based therapeutic strategies, advanced research tools, and ethical concerns. Attendees were able to interact with senior investigators on a one-to-one basis in an informal environment. Nonfaculty students were eligible for partial stipends, depending on stated need. Applications were invited from medical and graduate students, postdoctoral fellows, and faculty.

This course was supported with funds provided by the Lustgarten Foundation and Pancreatic Cancer Action Network.

PARTICIPANTS

Abel, E., Ph.D., University of Michigan, Ann Arbor
Arnes, L., Ph.D., Columbia University, New York
Bader, M., Ph.D., Pancreatic Cancer Action Network, Manhattan Beach, California

Bernard, V., M.D./Ph.D., MD Anderson Cancer Center, Houston, Texas
Bryant, K., Ph.D., University of North Carolina, Chapel Hill
Direna Curbelo, D., Memorial Sloan Kettering Cancer Center, New York
Dudgeon, C., Ph.D., Rutgers University, New Brunswick, New Jersey
DuFort, C., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Halbrook, C., Ph.D., Mayo Clinic, Jacksonville, Florida
Hunter, J., Ph.D., University of Texas Southwestern Medical Center, Dallas
Leal, A.S., Ph.D., Geisel School of Medicine at Dartmouth, Hanover, New Hampshire
Lin, C.-Y., Ph.D., University of Houston, Texas
Livshits, G., Ph.D., Memorial Sloan Kettering Cancer Center, New York
Mielgo Iza, A., Ph.D., University of Liverpool, United Kingdom
Moir, J., Ph.D., Newcastle University, United Kingdom
Rajurkar, M., Ph.D., Massachusetts General Hospital, Boston, Massachusetts
Ridinger, M., Ph.D., Salk Institute, San Diego, California
Roth, S., M.D., Technical University Munich, Germany
Schanne, D., M.D., Massachusetts General Hospital, Boston
Skalicky, A., M.D., Teaching Hospital in Pilsen, Czech Republic
Wang, D., M.D./Ph.D., Weill Cornell Medical College, New York
Zhang, S.Q., M.D., University of Chicago, Illinois
Zhang, S.Y., M.D., Emory University, Atlanta, Georgia

SEMINARS

Tempero, M., University of California, San Francisco: Title unknown.
Simeone, D., University of Michigan, Ann Arbor: Diagnostic evaluation and surgical management of pancreatic cancer.
Leach, S., Memorial Sloan Kettering Cancer Center, New York: Mouse models of pancreatic cancer.
Tuveson, D., Cold Spring Harbor Laboratory: Organoid models of pancreatic cancer.
Hebrok, M., University of California, San Francisco: Title unknown.
Crawford, H., University of Michigan, Ann Arbor: Developmental pathways in pancreatic cancer.
Iacobuzio-Donahue, C., Memorial Sloan Kettering Cancer Center, New York: Pancreatic cancer genetics.
Weaver, V., University of California, San Francisco: Extrinsic and intrinsic force modulated PDAC progression.
Fearon, D., Cold Spring Harbor Laboratory: Escape from immune recognition by pancreatic adenocarcinoma.

Kimmelman, A., Dana-Farber Cancer Institute, Boston, Massachusetts: Identifying metabolic dependencies in pancreatic cancer.
Wolpin, B., Dana-Farber Cancer Institute, Boston, Massachusetts: Pancreatic adenocarcinoma and host systemic metabolism.
Bar-Sagi, D., New York University Langone Medical Center, New York: The bad drinking habits of pancreatic tumors.
Ting, D., Massachusetts General Hospital, Boston: Pancreatic cancer liquid biopsies.
Kelly, K., University of Virginia, Charlottesville: Imaging in pancreatic cancer.
Der, C., University of North Carolina, Chapel Hill: Targeting KRAS: Know your enemy.
Draetta, G., MD Anderson Cancer Center, Houston, Texas: Developing a better understanding of metabolic dependencies in pancreatic cancer.
LoRusso, P., Yale University, New Haven, Connecticut: Title unknown.
Hollingsworth, T., University of Nebraska, Lincoln: Title unknown.
This laboratory/lecture course was intended for researchers at all levels, from beginning graduate
students through established primary investigators, who wanted to use Drosophila as an exper-
imental system for nervous system investigation. The 3-week course was designed to introduce
students to a wide variety of topics and techniques, including the latest approaches for 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 ner-
vous system development or function or focused on state-of-the-art techniques and approaches
in Drosophila neuroscience. 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 on inquiry-based projects, utilizing
the different morphological and physiological measurements and behavioral paradigms learned
through the course. This included molecular-genetic analyses, immunocytochemistry, recording of activity using electrophysiology and genetically encoded calcium indicators, and optogenetic and thermogenetic control of neural activity, as well as numerous quantitative behavioral measures. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the neural basis of behavior in *Drosophila*.

The course was supported by funds provided by the National Institute on Drug Abuse, the National Science Foundation, and the Howard Hughes Medical Institute.

**PARTICIPANTS**

Aponte Santiago, N., B.S., Massachusetts Institute of Technology, Cambridge  
Auer, T., Ph.D., University of Lausanne, Switzerland  
Benjamin, J., B.S., Texas A&M University, College Station  
Filosevic, A., M.S., University of Rijeka, Croatia  
Franco, L., Ph.D., Instituto de Investigaciones en Biodiversidad y Medioambiente, CONICET, Argentina  
Golovin, R., Ph.D., Vanderbilt University, Nashville, Tennessee  
Grossier, J.-P., Ph.D., New York University, New York  
Hill, A., Ph.D., Washington University, St. Louis, Missouri  
Landayan, D., B.S., University of California, Merced  
Ly, S., B.S., University of Pennsylvania, Philadelphia  
Satterfield, L., B.S., University of California, San Diego, La Jolla  
Wang, I., Ph.D., Stanford School of Medicine, California

**SEMINARS**

Cardona, A., HHMI/Janelia Research Campus, Ashburn, Virginia: Electron microscopy approaches to mapping *Drosophila* circuits.  
Prinz, A., Emory University, Atlanta, Georgia: Computational modeling of *Drosophila* neurons. Variability, stability, and homeostasis in neuronal circuits.  
Rogulja, D., Harvard Medical School, Cambridge, Massachusetts, and Masck, P., University of Nevada, Reno: Motivated behaviors in *Drosophila* II: Sleep and feeding.  
Kravitz, E., Harvard University, Cambridge, Massachusetts: Neuromodulatory systems and aggression in *Drosophila*.  
Levitan, E., University of Pittsburgh, Pennsylvania, and Rolls, M., Penn State University, State College, Pennsylvania: Microscopy, dense core vesicles, neurosecretion, neuronal polarity.  
Turner, G., Cold Spring Harbor Laboratory: Physiology of mushroom body neurons.  
Krupp, J., and Schneider, J., University of Toronto, Mississauga, Ontario, Canada: Social behavior and tracking in *Drosophila*.  
Dubnau, J., Cold Spring Harbor Laboratory: Motivated behaviors in *Drosophila* I: Learning and memory.

Kaun, K., Brown University, Providence, Rhode Island: Modeling complex brain disorders in *Drosophila*: Addiction as a case study.  
O’Connor-Giles, K., University of Wisconsin, Madison: Genome editing: Fly CRISPR development and use.  
Griffith, L., Brandeis University, Waltham, Massachusetts: How sleep influences memory in the *Drosophila* brain.  
Zwart, M., HHMI/Janelia Research Campus, Ashburn, Virginia: Central nervous system physiology I.  
Louis, M., EMBL Center for Genomic Regulation, Spain: Larval sensory systems and behavior.  
Daniels, R., University of Wisconsin, Madison: Neuromuscular junction physiology I. Neuromuscular junction physiology II.  
Pulver, S., University of St. Andrews, Scotland, United Kingdom: Optical imaging of central pattern generator output.  
Harbison, S., NHLBI, National Institutes of Health, Bethesda, Maryland: Quantitative trait loci.  
Frontiers and Techniques in Plant Science

June 26–July 16

INSTRUCTORS  
M. Johnson, Brown University, Providence, Rhode Island  
S.-H. Shiu, Michigan State University, E. Lansing  
M. Timmermans, University of Tübingen, Germany

CO-INSTRUCTOR  
N. Provart, University of Toronto, Ontario, Canada

ASSISTANTS  
J. Forcina, Brown University, Providence, Rhode Island  
U.-S. Lee, University of Pennsylvania, Philadelphia  
J. Lloyd, Michigan State University, E. Lansing  
N. Panchy, Michigan State University, E. Lansing

This course provided an intensive overview of topics in plant genetics, physiology, biochemistry, development, and evolution and hands-on experiences in molecular, analytical, computational, and 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. The course was designed for scientists with some experience in molecular techniques or in plant biology who wish to work with plants using the latest technologies. The course consisted of a vigorous lecture series, hands-on laboratories, informal discussions, and social activities designed to foster an intense but enjoyable learning environment. The instructors and a stellar group of speakers who are acknowledged leaders in their fields presented up-to-the-moment research and led laboratory sessions on a wide range of topics in plant research.
The seminar series included plant morphology and anatomy, development, evolution, light and circadian biology, hormones, small RNAs and epigenetic inheritance, biotic and abiotic interactions, biochemistry and physiology, and applications addressing current agronomic and biotechnological problems. Speakers provided expert overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions provided exposure to cutting-edge experimental and computational techniques currently used in plant research. These included approaches for studying plant development, transient gene expression, applications of fluorescent proteins, genome editing, and chromatin immunoprecipitation. Students also gained hands-on experience with computational tools and environments for genome assembly, transcriptomics, construction of gene regulatory networks, identification of quantitative trait loci, mapping by sequencing, and mathematical modeling of development and hormone action. The course also included several short workshops on important themes in plant research. Throughout the course, students interacted individually and informally with the speakers to further enrich the learning experience.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Farr, K., B.S., University at Buffalo, New York
Gates, L., B.S., University of California, San Diego, La Jolla
Khakhar, A., B.S., University of Washington, Seattle
Kumar, K., B.S., Michigan Technological University, Houghton
Kumar, S., B.Sc., University of Nebraska, Lincoln
Lee-Parsons, C., B.S., Northeastern University, Boston, Massachusetts
Lolle, S., B.Sc., University of Copenhagen, Denmark
Maher, M., B.S., University of Minnesota, Minneapolis
Mayer, J., B.S., University of Nevada, Reno
McNinch, C., B.S., Iowa State University, Ames
Nguyen, N., B.S., University of Missouri, Columbia
Nummert, G., B.S., Tallinn University of Technology, Tallinn, Estonia
Potok, M., B.S., University of California, Los Angeles
Stanimirovic, S., B.S., University of Copenhagen, Denmark
Wahrenburg, Z., B.S., DePaul University, Chicago, Illinois

SEMINARS

Kuhlemeier, C., University of Bern, Switzerland: Phyllotaxis: A quantitative developmental problem.
Voytas, D., University of Minnesota, Falcon Heights: Precise genome engineering with sequence-specific nucleases.
Weigel, D., Max Planck Institute for Development Biology, Tübingen, Germany: Molecular evolution.
Schemske, D., Michigan State University, E. Lansing: Ecological genetics of adaptation.
Kramer, E., Harvard University, Winchester, Massachusetts: How-to-EvoDevo: Pitfalls and considerations in comparative development. Aquilegia as a test case for developing new model systems.
Howe, G., Michigan State University, E. Lansing: Plant–insect interactions.
Bailey-Serres, J., University of California, Riverside: Abiotic stress response.
Kang, J., University of Northern Iowa, Cedar Falls: Development of leaf shape.
Law, J., The Salk Institute for Biological Studies, La Jolla, California: Chromatin modifications and epigenetics.
Malloof, J., University of California, Davis: Light signaling.
Johnson, M., Brown University, Providence, Rhode Island: Molecular dialogs between pollen and pistil: Start pollen tube growth.
Timmerman, M., University of Tübingen, Germany: Leaf polarity.
Provart, N., University of Toronto, Canada: Bioinformatic tools in plant research.
Sage, R., University of Toronto, Canada: Ecological genetics of adaptation.
Cutler, S., University of California, Riverside: Chemical genomics.
Shiu, S.-H., Michigan State University, E. Lansing: Genome evolution.
Brady, S., University of California, Davis: Regulation of root development.
Harmer, S., University of California, Davis: Circadian rhythms.
Paszkowski, U., University of Cambridge, United Kingdom: Molecular genetics of plant fungal endosymbiosis.
Advanced Techniques in Molecular Neuroscience

June 30–July 16

INSTRUCTORS
C. Lai, Indiana University, Bloomington
J. Loturco, University of Connecticut, Storrs
A. Schaefer, Icahn School of Medicine, Mount Sinai, New York

ASSISTANT
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 CRISPR genome editing and RNAi approaches for regulating the expression of specific genes in neurons; practical exercises in gene delivery systems including mammalian cell infection and transfection and electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use, and design of BAC transgenic vectors; RT-PCR analyses; assays of chromatin and chromatin structure in neurons; and mRNA isolation from specified neural subtypes by TRAP.

This course was supported with major funding provided by the National Institute of Mental Health, Helmsley Charitable Trust, and the Howard Hughes Medical Institute.
PARTICIPANTS

Arroyo, E., B.S., University of California, Los Angeles
Clements, R., B.A., Vollum Institute, Portland, Oregon
de la Fuente, V., Ph.D., University of Buenos Aires, Argentina
Fleming, E., B.A., Duke University, Durham, North Carolina
Harris, L., B.S., University of Queensland, St. Lucia, Australia
Jennings, K., B.S., University of California, Berkeley
Kottick, A., M.S., College of William and Mary, Williamsburg, Virginia
Li, L., M.S., A.I. Virtanen Institute for Molecular Sciences, UEF, Kuopio, Finland
Melas, P., Ph.D., CMM, Karolinska Institutet, Stockholm, Sweden
Ramirez, A., Ph.D., Weill Cornell Medical College, New York
Rueppell, M., M.Sc., Bernstein Center Freiburg/Freiburg University, Germany
Stauffer, W., Ph.D., University of Cambridge, United Kingdom
Tee, W.-W., Ph.D., HHMI/New York University School of Medicine, New York
Tonner, K., B.A., University of Minnesota, Minneapolis
Torz, L., M.S., Copenhagen University, Denmark
Zheng, D.-J., M.S., The University of Texas, Austin

SEMINARS

Schaefer, A., Icahn School of Medicine, Mount Sinai, New York: Epigenetics and miRNA.
Barres, B., Stanford University School of Medicine, California: What do astrocytes do? Multi-spectral image analysis.
Darnell, B., HHMI/Rockefeller University, New York: Applying advanced techniques in molecular neuroscience to the human brain.
Maher, B., Lieber Institute for Brain Development/Johns Hopkins Medical Campus, Baltimore, Maryland: Methods and uses for neuronal transgenesis by in utero electroporation.
Luikart, B., Geisel School of Medicine at Dartmouth, Lebanon, New Hampshire: Lentivirus production and uses in neuroscience.
Lai, C., Indiana University, Bloomington: Application of BAC technology in neuroscience.
Cai, D., University of Michigan, Arbor Harbor: New and improved brain-bow technologies.
Borrelli, E., University of California, Irvine: Dopamine and the modulation of neuronal circuitries.
Mandoiu, I., University of Connecticut, Storrs: Computational approaches to analyzing single-cell transcriptomics data.
Eberwine, J., University of Pennsylvania Perelman School of Medicine, Philadelphia: Single-cell transcription variation: Surprises from human and mouse studies.
Dougherty, J., Washington University School of Medicine, St. Louis, Missouri: RNA isolation and profiling by BAC-TRAP.
LoTurco, J., University of Connecticut, Storrs: Course introduction/module introduction. CRISPR/Cas9 technology.
Haas, K., University of British Columbia, Canada: Single-cell electroporation and imaging.
Zador, T., Cold Spring Harbor Laboratory: Sequencing the Connectome.
Gradinaru, V., California Institute of Technology, Pasadena: Clarity and optogenetics.
Vision: A Platform for Linking Circuits, Perception, and Behavior

July 7–20

INSTRUCTORS  
F. Briggs, Geisel School of Medicine at 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 in order 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 with funds provided by the National Eye Institute, Helmsley Charitable Trust, and the Howard Hughes Medical Institute.

PARTICIPANTS

Alexander, P., Ph.D., University of California, Davis
Beier, C., Ph.D., University of California, Santa Cruz
Dipoppa, M., Ph.D., University College London, United Kingdom
Drews, M., Ph.D., Max-Planck Institute for Neurobiology, Martinsried, Germany
Drinnenberg, A., Ph.D., Friedrich Miescher Institute Basel, Switzerland
Gamlin, C., B.A., University of Washington, Seattle
Ghodrati, M., Ph.D., Monash University, Melbourne, Australia
Gribizis, A., B.A., Yale University, New Haven, Connecticut
Hartstein, K., Ph.D., Dartmouth College, Hanover, New Hampshire
Huh, C., Ph.D., Stanford University, California
Kay, R., Ph.D., Children’s National Health System, Washington, D.C.
Koren, D., M.D./Ph.D., University of Chicago, Illinois
Pyle, L., M.S., University of Louisville School of Medicine, Louisville, Kentucky
Ramamurthy, M., M.S., University of Massachusetts, Boston
Sajdak, B., Ph.D., Medical College of Wisconsin, Milwaukee
Salay, L., Ph.D., University of California, San Diego
Schmidt, B., B.A., University of Washington, Seattle
Seabrook, T., Ph.D., University of California, San Diego
Seemiller, E., Ph.D., Indiana University, Bloomington
van Opheusden, B., M.S., New York University, New York
Wal, A., Ph.D., University of Tübingen, Germany
Wang, S., B.S., Brandeis University, Waltham, Massachusetts
Wekselblatt, J., Ph.D., University of Oregon, Eugene
Wilson, A., Ph.D., Duke University, Durham, North Carolina
Zavitz, E., Ph.D., Monash University, Clayton, Australia

SEMINARS

Huberman, A., University of California, San Diego: Visual system overview.
Briggs, F., Geisel School of Medicine at Dartmouth, Hanover, New Hampshire: Visual system overview.
Roska, B., Friedrich Miescher Institute, Basel, Switzerland: Circuit motifs for generating specialized receptive fields.
Dunn, F., University of California, San Francisco: Gain control in the mammalian retina/development and disassembly of the visual system’s first synapse.
Solomon, S., University College London, United Kingdom: Comparative physiology of early visual pathways.
Guido, W., University of Louisville, Kentucky: Dissecting circuitry in mouse visual thalamus.
Hirsch, J., University of Southern California, Los Angeles: Receptive field transformations.
Usrey, W.M., University of California, Davis: Functional properties of neural circuits for vision.
Fitzpatrick, D., Max-Planck Florida Institute for Neuroscience, Jupiter: Visualizing cortical maps and their development with cellular and synaptic resolution.
Callaway, E., The Salk Institute, La Jolla, California: Functional architecture and cortical specializations.
Hofer, S., University of Basel, Switzerland: Functional architecture and cortical specializations.
Desplan, C., New York University, New York: Color vision.
Horwitz, G., University of Washington, Seattle: Color vision.
Pearson, R., University College London, United Kingdom: Cellular-based strategies for repairing the diseased retina.
Chichilnisky, E.J., Stanford University, California: Restoring vision.
Becker, S., National Institutes of Health, Bethesda, Maryland: The NEI audacious goals initiative: Catalyzing neurodegenerative medicine.
Niell, C., University of Oregon, Eugene: The behavioral state, locomotion, and visual processing.
Churchland, A., Cold Spring Harbor Laboratory: State-dependent influences on visual processing.
Rust, N., University of Pennsylvania, Philadelphia: Receptive field transformations.
Maunsell, J., University of Chicago, Illinois: Cortical computations for vision and attention.
Wandell, B., Stanford University, California: Cortical computations: Maps and modeling.
Krauzlis, R., National Institutes of Health, Bethesda, Maryland: Subcortical control of eye movements and attention.
Gottlieb, J., Columbia University, New York: Eye movements and attention.
Tsao, D., California Institute of Technology, Pasadena: Image recognition and perception.
Gallant, J., University of California, Berkeley: Natural vision.
Proteomics

July 14–27

INSTRUCTORS
M. Cilia, Cornell University, Ithaca, New York
I. Cristea, Princeton University, New Jersey
K. Medzihradsky, University of California, San Francisco
D. Pappin, Cold Spring Harbor Laboratory

ASSISTANTS
M. Connolly, Cold Spring Harbor Laboratory
M. Crow, Princeton University, New Jersey
T. Greco, Princeton University, New Jersey
A. Kruse, Cornell University, Ithaca, New York
K. Rivera, Cold Spring Harbor Laboratory
F. Robison, Cornell University, Ithaca, New York
J. Wilson, Cold Spring Harbor Laboratory

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. 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 nano HPLC 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. Students performed posttranslational modification analysis: Phosphopeptides were enriched from identical sources exposed to different stimuli, and the resulting mixtures were subjected to multiple LC/MS/MS analyses. Results obtained by different LC/MS experiments and phosphorylation data as the result of different stimulations were compared. Special attention was given to accurate site assignments. 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.

Financial support was provided by the National Institute of Child Health and Human Development and Helmsley Charitable Trust.

PARTICIPANTS

Brent, M., Ph.D., Washington University, St. Louis, Missouri
Fraser, A., B.S., Roslin Institute, Edinburgh, United Kingdom
Kawatkar, A., M.S., AstraZeneca, Waltham, Massachusetts
Lavoie, J., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland
Lebedeva, O., Ph.D., Belgorod State National Research University, Belgorod, Russia
Lewinski, M., Ph.D., University of California, San Diego
Mannix, K., B.S., Yale University, New Haven, Connecticut
Moore, R., Ph.D., National Institutes of Health, Hamilton, Montana
Nowakowski, A., Ph.D., Northwestern University, Evanston, Illinois
Quijada, J., Ph.D., Northeastern University, Boston, Massachusetts
Quinlan, M., B.S., Vanderbilt University, Nashville, Texas
Ree, R., M.Sc., University of Bergen, Bergen, Norway
Tamir, T., B.S., University of North Carolina, Chapel Hill
Wilczewski, C., B.S., University of North Carolina, Chapel Hill
Yates, A., B.S., The Ohio State University, OARDC, Wooster
Zheng, X.-F., Ph.D., Dana-Farber Cancer Institute, Boston, Massachusetts

SEMINARS

Pappin, D., Cold Spring Harbor Laboratory: Introduction to protein and peptide identification.
Conlon, F., University of North Carolina, Chapel Hill: Functional investigation of the cardiac interactomes.

Medzihradszky, K., University of California, San Francisco: How to interpret/evaluate MS/MS spectra-focus on PTM assignments.
Bereman, M., North Carolina State University, Apex: Introduction to mass spectrometry via WebEx.
Greco, T., Princeton University, New Jersey: Computational approaches for constructing and analyzing protein interaction networks.
This course was designed for students, postdocs, and principal investigators who had recently ventured into the exciting area of gene regulation. The course focused on state-of-the-art strategies and techniques used in the field. Emphasis was placed on both in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, isolated transcription factor complexes, performed in vitro transcription reactions, and measured RNA levels using primer extension. In addition, students learned techniques for assembling recombinant chromatin and biophysical methods to assay the activity of chromatin remodeling enzymes.

During the past few years, the gene regulation field has developed powerful in vivo approaches to study gene regulation. Students learned and 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 by the National Cancer Institute.

PARTICIPANTS

Afik, S., M.S., University of California, Berkeley
Bacon, C., B.S., University of Texas Southwestern Medical Center, Dallas
Cirak, S., Ph.D., Children’s National Medical Center, Washington, D.C.
Engelhardt, J., M.Sc., University of Leipzig, Germany
Eskla, K.-L., M.S., University of Tartu, Estonia
Heier, C., Ph.D., Children’s National Medical Center, Washington, D.C.
Hluchy, M., M.S., CEITEC Masaryk University, Brno, Czech Republic
Kaminski, J., B.A., University of California, Berkeley
Koney-Kwaku Koney, N., M.Phil., University of North Dakota, Grand Forks
Palfy, M., M.Sc., Max-Planck Institute, Dresden, Germany
Rezvan, A., M.S., Emory University, Atlanta, Georgia
Rister, J., Ph.D., New York University, New York
Sinkoe, A., B.S., Rensselaer Polytechnic Institute, Troy, New York
Stromme, C., Ph.D., Biotech Research and Innovation, Copenhagen, Denmark
Tachtsidi, A., M.S., Institut Pasteur, Paris, France
Xie, P., M.S., University of Texas, Dallas, Richardson

SEMINARS

Stark, A., Institute of Molecular Pathology, Vienna, Austria: Systems biology of regulatory motifs.
Bentley, D., University of Colorado, Aurora: Cotranscriptional mRNA processing.
Goodrich, J., University of Colorado, Boulder: Fundamental of RNA polymerase transcription.
Rinn, J., Harvard University, Cambridge, Massachusetts: Linking RNA to human health and disease.
Whetstine, J., Harvard University, Charlestown, Massachusetts: Histone demethylases in health and disease.
Di Croce, L., ICREA and Centre for Genomic Regulation, Barcelona, Spain: Developmental control of chromatin modifying enzymes.
Levine, M., Princeton University, New Jersey: Definition of cell fate and behavior through enhancers.
Robash, M., Brandeis University, Waltham, Massachusetts: Circadian rhythms, behavior, and gene expression.
Silver, P., Harvard Medical School, Boston, Massachusetts: Designing biology for a healthy world.
Kingston, R., Massachusetts General Hospital, Harvard Medical School, Boston: Noncoding RNAs and polycomb gene silencing.
Buratowski, S., Harvard Medical School, Boston, Massachusetts: Pol II code and coupling of transcription with chromatin.
Bickmore, W., MRC Human Genetics Unit, Edinburgh, United Kingdom: Spatial organization of the nucleus.
Yeast Genetics and Genomics

July 21–August 10

INSTRUCTORS
G. Brown, University of Toronto, Ontario, Canada
M. Dunham, University of Washington, Seattle
M. Gartenberg, Robert Wood Johnson Medical School, Piscataway, New Jersey

ASSISTANTS
M. Borrie, Robert Wood Johnson Medical School, Piscataway, New Jersey
D. Gallo, University of Toronto, Ontario, Canada
C. Smukowski Heil, 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. Students learned array-based methods, next-generation sequencing, and genome-based methods of analysis facilitated by the yeast genome sequence, 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, metabolic engineering using CRISPR, 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 (including SGA). Students were immersed in yeast genomics and performed and interpreted experiments using DNA arrays, whole-genome sequencing, and multiplexed DNA barcode sequencing. Students gained first-hand experience in modern cytological approaches such
as epitope tagging and imaging yeast cells using GFP–protein fusions and a variety of fluorescent indicators for different 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 by the National Science Foundation and Helmsley Charitable Trust.

PARTICIPANTS

Abdul-Rahman, F., M.S., New York University, New York
Colon-Cesario, M., Ph.D., University of Puerto Rico, Humacao
Docter, B., B.S., University of Michigan, Ann Arbor
Fisher, K., M.S., Lehigh University, Bethlehem, Pennsylvania
Gerosa, L., Ph.D., Harvard University, Cambridge, Massachusetts
Inda, M.E., Ph.D., Facultad de Ciencias Bioquímicas y Farmacéuticas, Rosario, Argentina
Kalapis, D., M.A., Biological Research Centre, Szeged, Hungary
Kayser, J., Ph.D., University of California, Berkeley
Lhuillier-Akakpo, M., Ph.D., French Atomic Energy Commission, Fontenay-aux-Roses, France
Miller, D., B.S., New York University, New York
Pandey, A., Ph.D., Louisiana State University, Baton Rouge
Penitz, J., B.S., Georgia Institute of Technology, Atlanta
Ting, A., Ph.D., Massachusetts Institute of Technology, Boston
Xu, H., Ph.D., BASF, Tarrytown, New York
Yeung, R., B.Sc., New York University, New York
Zies, D., Ph.D., University of Mary Washington, Fredericksburg, Virginia

SEMINARS

Caudy, A., University of Toronto, Ontario, Canada: Many ways to rot a fig: New biochemistry in yeast from discovery metabolomics.
Baryshnikova, A., Princeton University, New Jersey: High-throughput genetic interaction analysis via synthetic genetics array (SGA).
Nislow, C., University of British Columbia, Vancouver, Canada: The year deletion collection perspective: Molecular barcodes past, present, and future.
Lew, D., Duke University, Durham, North Carolina: Polarity establishment, or how yeast cells know front from back.
Fowler, D., University of Washington, Seattle: Deep mutational scanning to study protein properties and evolution.
Haber, J., Brandeis Harvard, Waltham, Massachusetts: Uses and abuses of HO endonuclease: Analyzing DNA repair and the DNA-damage checkpoint.
Wolfe, K., Trinity College Dublin, United Kingdom: The world beyond the S. cerevisiae model: Different genomes different biology.
Hochstrasser, M., Yale University, New Haven, Connecticut: End of the road: Protein destruction and the ubiquitin-proteasome pathways.
Rose, M., Princeton University, New Jersey: Regulation of mating and meiosis by Kar4p.
Hampsey, M., Rutgers University, Piscataway, New Jersey: Genetic suppression: Rehabilitation of a translation until defect leads to a pathway from transcription initiation to nuclear mRNA export.
Silver, P., Harvard Medical School, Boston, Massachusetts: Designing biology for a healthy world.
Nash, R., Stanford University, Palo Alto, California: A hands-on tour of SGD.
Levy, S., Stony Brook University, New York: Evolutionary dynamics of one million barcoded lineages.
Gasser, S., Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland: Chromatin dynamics and spatial aspects of DNA repair.
Imaging Structure and Function in the Nervous System

July 21–August 10

INSTRUCTORS

F. Albeanu, Cold Spring Harbor Laboratory
M. Orger, Champalimaud Foundation, Lisbon, Portugal
L. Palmer, University of Melbourne, Victoria, Australia
P. Tsai, University of California, San Diego, La Jolla
J. Waters, Allen Institute for Brain Sciences, Seattle, Washington
K. Zito, University of California, Davis

ASSISTANTS

U.L. Boehm, Paris School of Neuroscience (ENP), Paris, France
O. Braubach, Korea Institute of Science and Technology, Seoul, Seongbuk-gu, Republic of Korea
P. Garcia da Silva, Cold Spring Harbor Laboratory
M. Gremey, New York University Langone Medical Center, New York
I. Stein, University of California, Davis
N. Takahashi, Humboldt University of Berlin, Germany

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular structure, the course examined a variety of
molecular probes of cell function, including calcium-sensitive dyes, and optogenetic and photoactivatable molecules. Particular weight was given to multiphoton laser-scanning microscopy and to biological fluorophores, especially GFP and its variants. A spectrum of neural and cell biological systems were used, including living mice, zebrafish, and brain slices.

Applicants were expected to have 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 in part by the Helmsley Charitable Trust, Howard Hughes Medical Institute, and Burroughs Wellcome Fund.

PARTICIPANTS

Bohorquez, D., Ph.D., Duke University, Durham, North Carolina
Ford, M.F., Diplom., University College London, United Kingdom
Hildebrand, D., B.S., Harvard Medical School, Cambridge, Massachusetts
La Chioma, A., M.S., Max Planck Institute of Neurobiology, Martinsried, Germany
Li, Y., Ph.D., Harvard University, Cambridge, Massachusetts
Morimoto, M., M.S., HHMI/Janelia Farm Research Campus, Ashburn, Virginia
O’Neill, P., Ph.D., Columbia University, New York

Pattadkal, J., M.S., University of Texas, Austin
Pi, H.J., Ph.D., Cold Spring Harbor Laboratory
Prigge, M., Ph.D., Weizmann Institute of Science, Rehovot, Israel
Sternberg, J., B.S., Institut du Cerveau et de la Moelle épinière, Paris, France
Takkala, P., B.S., The Hospital for Sick Children, Toronto, Canada
Truszkowski, T., Ph.D., Brown University, Providence, Rhode Island
Wilson, D., Ph.D., Max-Planck Florida Institute for Neuroscience, Jupiter

SEMINARS

Wyart, C., Institut du Cerveau et de la Moelle épinière, Paris, France: In vivo optogenetics and circuits analysis in zebrafish spinal cord.
Dombeck, D., Northwestern University, Evanston, Illinois: Awake imaging in hippocampus.
Albeau, F., Cold Spring Harbor Laboratory: Intrinsic imaging.
Helmchen, F., Brain Research Institute, Zurich, Switzerland: 2P microscopy: Lasers.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Confocal microscopy. Incoherent emission pt source, PSF/Airy pattern, Fourier/OTF, aberrations.
Mertz, J., Boston University, Boston, Massachusetts: Contrast: Phase, DIC, De Senamont DIC, Dodt tube.
Zito, K., University of California, Davis: Photolysis.
Deisseroth, K., Stanford University, California: Optogenetics: Recent advances/CLARITY.
Looger, L., Howard Hughes Medical Institute, Ashburn, Virginia: XFPs/GECIs/GEVIs.
Paninski, L., Columbia University, New York: Spike extraction.
Palmer, L., University of Melbourne, Victoria, Australia: Organic calcium indicators.
Orger, M., Champalimaud Foundation, Lisbon, Portugal: Light-sheet microscopy/whole-animal imaging in zebrafish.
Ji, N., HHMI/Janelia Farm Research Campus, Ashburn, Virginia: Superresolution imaging—Structured illumination, PALM, STED. Deep imaging/adaptive optics.
Waters, J., Harvard Medical School, Boston, Massachusetts: Noise and detectors.
Yasuda, R., Max-Planck Florida Institute for Neuroscience, Jupiter: FRET and FLIM.
Dieudonné, S., CNRS–Laboratoire de Neurobiologie, Paris, France: AODs and random-access multiphoton imaging.
Oertner, T., Center for Molecular Neurobiology Hamburg, Germany: Optogenetics: Introduction.
Denk, W., Max-Planck Institute of Neurobiology, Martinsried, Germany: Block-face EM/connectome.
Neural Data Science

July 23–August 2

INSTRUCTORS

M. Reimers, Michigan State University, East Lansing
P. Wallisch, New York University, New York

Computational approaches to neuroscience will produce important advances in our understanding of neural processing. Prominent success will come in areas where strong inputs from neurobiological, behavioral, and computational investigation can interact. The theme of the course was that an understanding of the computational problems, the constraints on solutions to these problems, and the range of possible solutions can help guide research in neuroscience. Through a combination of lectures and hands-on experience with MATLAB-based computer tutorials and projects, this intensive course examined visual information processing from the retina to higher cortical areas, spatial pattern analysis, motion analysis, neuronal coding and decoding, attention, and decision-making.

This course was supported with funds provided by the Helmsley Charitable Trust.

PARTICIPANTS

Bonnevie, T., Ph.D., Norwegian University for Science & Technology, Trondheim, Norway
Chen, X., Ph.D., Harvard University, Cambridge, Massachusetts
Ganglberger, F.J., M.S., VRVis, Vienna, Austria
Gao, Y., Ph.D., University of Tennessee Health Science Center, Memphis
Hill, J.L., Ph.D., Lieber Institute for Brain Development, Baltimore, Maryland
Hong, Y.K., Ph.D., Columbia University, New York
Inman, C.S., Ph.D., Emory University, Atlanta, Georgia
Kollias, P., Ph.D., Princeton University, New Jersey
Mani, A., Ph.D., Northwestern University, Evanston, Illinois
Mihali, A.L., Ph.D., New York University, New York
Parthasarathy, A., M.S., National University of Singapore, Singapore
Patel, R.R., Ph.D., Stark Neuroscience Research Institute, Indianapolis, Indiana
Sidorov, M., Ph.D., University of North Carolina, Chapel Hill
Valley, M.T., Ph.D., Allen Institute for Brain Science, Seattle, Washington
Vincent, J.L., Ph.D., Harvard Medical School, Boston, Massachusetts

SEMINARS

Reimers, M., Michigan State University, East Lansing: Visualizing state space: Dimensionality reduction methods. Introduction to data analysis issues for neuroimaging.
Osborne, L., University of Chicago, Illinois: Analyzing single electrode data with information theory.
Helmchen, B.F., Brain Research Institute: Introduction to calcium imaging.
Pillow, J., Princeton University, New Jersey: Encoding models using GLM. Encoding models using GLM (exercise).
Kording, K., Northwestern University, Evanston, Illinois: Approaches to decoding population data.

Approaches to decoding population data (exercise).
Paper writing.
Simon, J., University of Maryland, Baltimore: Spectral analysis for MEG/EEG. Spectral analysis for MEG/EEG (exercise).
Cohen, M., University of Amsterdam, The Netherlands: Applications of spectral analysis.
Deneve, S., Ecole Normale Superieure, Paris, France: How can neural networks represent and compute efficiently? What does efficient coding predict for neural data?
Kriegeskorte, N., University of Cambridge, United Kingdom: Testing models with functional MRI data. Testing models with functional MRI data (Exercise).
Freeman, J., HHMI/Janelia Farm Research Campus, Ashburn, Virginia: Infrastructure for big neuroimaging data.
Synthetic Biology

July 27–August 10

INSTRUCTORS
J. Dueber, University of California, Berkeley
M. Dunlop, University of Vermont, Burlington
K. Haynes, Arizona State University, Tempe
J. Lucks, Cornell University, Ithaca, New York
P. Peralta-Yahya, Georgia Institute of Technology, Atlanta
L.S. Qi, Stanford University, California

ASSISTANTS
J. Chappell, Cornell University, Ithaca, New York
R. Davis, Arizona State University, Tempe
L. Latimer, University of California, Berkeley
N. Rossi, University of Vermont, Burlington
E. Yasi, Georgia Institute of Technology, Atlanta
D. Zhao, Stanford University, California

Synthetic biology is a discipline wherein living organisms are genetically programmed to carry out desired functions in a reliable manner. This field takes inspiration from our ever-expanding ability to measure and manipulate biological systems and the philosophical reflections of Schrödinger and Feynman that physical laws were used to describe and rationally engineer biology to accomplish useful goals. After all, cells are the world’s most sophisticated chemists, and their ability to learn to adapt to changing environments offers enormous potential for solving modern engineering challenges. Nonetheless, biological systems are noisy, massively interconnected, and nonlinear, and they have not evolved to be easily engineered. The grand challenge of synthetic biology was to reconcile the desire for a predictable, formalized biological design process with the inherent “squishiness” of biology.
The 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. It centered around an immersive laboratory experience. Here, students worked in teams to learn the practical and theoretical underpinnings of cutting-edge research in the area of synthetic biology. Broadly, we explored how cellular regulation—transcriptional, translational, post-translational and epigenetic—can be used to engineer cells to accomplish well-defined goals. Specific laboratory modules covered the following areas: cell-free transcription and translation systems, high-throughput cloning techniques, computational biology using ordinary differential equations and models, biosensor development for metabolic engineering, CRISPRi for gene regulation and changing cell phenotypes, and CRISPR for genome editing in mammalian cells to regulate synthetic genes and physical cell properties. Students first learned essential synthetic biology techniques in a 4-day boot camp and then rotated through research projects in select areas.

In addition, students interacted closely with a panel of internationally recognized speakers who gave students a broad overview of applications for synthetic biology, including renewable chemical production and therapeutics, the current state-of-the-art techniques, and case studies in human practices and socially responsible innovation.

This course was supported with funds provided by the National Institute of General Medical Sciences, Howard Hughes Medical Institute, Helmsley Charitable Trust, and National Science Foundation.

PARTICIPANTS

Alam, K., B.S., University of Missouri, Columbia
Bandyopadhyay, A., Ph.D., University of Kansas, Lawrence
Byrd, W., Ph.D., University of Utah, Salt Lake City
Cho, S., Ph.D., GIST College, Gwangju, South Korea
Coupland, K., Ph.D., Karolinska Institutet, Stockholm, Sweden
de Lange, O., Ph.D., University of Tübingen, Germany
Delepine, B., M.S., Institute of Systems and Synthetic Biology, Evry, France
Garcia Vanegas, K., M.S., Technical University of Denmark, Kongens Lyngby, Denmark
Hauser, D., B.S., The Princeton International School of Math and Science, Princeton, New Jersey

Jin, L., B.S., The Ohio State University, Columbus
Nutani, W., M.S., University of Wisconsin, Madison
Rodriguez-Paton, A., Ph.D., Universidad Politécnica de Madrid, Madrid, Spain
Shomar, H., M.Eng., Delft University of Technology, Delft, The Netherlands
St. Germain, B., M.S., Biogen, Cambridge, Massachusetts
Tanner, T., M.S., University of Utah, West Jordan
Wang, G., B.S., Courant Institute of Mathematical Sciences, New York

SEMINARS

Tullman-Ercek, D., University of California, Berkeley: Getting through the gatekeepers: Changing the selectivity of semipermeable cellular membranes.
Savage, D., University of California, Berkeley: Rapid construction of protein-based biosensors.
Tabor, J., Rice University, Houston, Texas: Engineering bacterial two-component signal transduction systems.
Franco, E., University of California, Riverside: Bottom-up construction of biomolecular materials.
Frow, E., Arizona State University, Tempe: CRISPR in context.
Khalil, M., Boston University/Wyss Institute at Harvard, Boston, Massachusetts: Naturally inspired engineering of biological systems.

Hillson, N., Harvard Medical School, Boston, Massachusetts: Synthetic biology informatics: Scalability and responsible innovation.
Silver, P., Harvard Medical School, Boston, Massachusetts: Designing biology for a healthy world.
Evans, S., Dow AgroSciences, Indianapolis, Indiana: Making synthetic biology work for plants.
Noireaux, V., University of Minnesota, Minneapolis: Cell-free transcription–translation: From gene circuits to self-assembly in a test tube.
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 neuroadaptative processes fundamental to addiction, withdrawal, craving, and relapse to drug use and to brain function, in general. A range of disciplines and topics were represented, including noninvasive brain imaging to identify drug targets and adaptive processes, neuroadaptative processes at the molecular and cellular level, neural networks and their modulation, the relevance of genotype to susceptibility and drug response, tolerance and
adaptation at the cellular level, and approaches to exploiting the daunting volume of information generated by neuroinformatics. This workshop provided an integrated view of current and novel research on neuroadaptive responses to addiction, fostered discussion on collaboration and integration, and provided critical information needed to construct a model of addiction as a disease and 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

Bobadilla, A.C., Ph.D., Medical University of South Carolina, Charleston
Cerrone, K., M.A., Academic Medical Center, Amsterdam, The Netherlands
Cohen-Gilbert, J., Ph.D., Harvard Medical School, Cambridge, Massachusetts
Fitzpatrick, C., Ph.D., University of Michigan, Ann Arbor
Guerri, L., Ph.D., National Institutes of Health, Rockville, Maryland
Healey, K., Ph.D., University of North Carolina, Chapel Hill
Juarez-Salinas, D., Ph.D., University of California, San Francisco
Madangopal, R., Ph.D., National Institute on Drug Abuse, Baltimore, Maryland
Neuhofer, D., Ph.D., Medical University of South Carolina, Charleston

O’Neill, B., Ph.D., New York University, New York
Paskulin, R., Ph.D., University of Ljubljana, Slovenia
Federsen, J., Ph.D., Uppsala University, Sweden
Rifkin, R., M.D./Ph.D., Icahn School of Medicine at Mount Sinai, New York
Schmidt, K., B.S., Emory University, Atlanta, Georgia
Smith, M., Ph.D., Oregon Health & Science University, Portland
Torres, O., Ph.D., Walter Reed Army Institute of Research, Silver Spring, Maryland
von Holstein-Rathlou, S., M.S., University of Copenhagen, Denmark
Yammine, L., Ph.D., University of Texas, Houston
Yuill, M., Ph.D., Penn State Hershey College of Medicine, Hershey, Pennsylvania

SEMINARS

Evans, C., University of California, Los Angeles: Opioids: Pain, stress and addiction.
Picciotto, M., Yale University, New Haven, Connecticut: Molecular basis of nicotine addiction.
Kenny, P., Mount Sinai Hospital, New York: Mechanisms of nicotine addiction.
Bonci, A., NIDA, National Institutes of Health, Bethesda, Maryland: From synaptic plasticity to a clinical study and a novel treatment against cocaine craving.

Belin, D., INSERM, Paris, France: Title unknown.
Mackie, K., Indiana University, Bloomington: Cannabinoids.
Wassum, K., University of California, Los Angeles: Behavioral process of addiction.
Kieffer, B., McGill University, Montreal, Canada: Opioid receptors in addiction and mood disorders: Mouse genetic approaches.
Sunahara, R., University of California, Davis: Title unknown.
Nestler, E., Mount Sinai Hospital, New York: Transcriptional and epigenetic mechanisms of addiction.
X-Ray Methods in Structural Biology

October 12–27

INSTRUCTORS
W. Furey, University of Pittsburgh, V.A. Medical Center, Pittsburgh, Pennsylvania
G. Gilliland, Janssen Research & Development LLC, Raritan, New Jersey
A. McPherson, University of California, Irvine
J. Pfugrath, Rigaku Americas Corporation, Texas

ASSISTANTS
A. Adamopoulos, University of California, Los Angeles
B. Hintze, Duke University Medical Center, Durham, North Carolina

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intense 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, cryocrystallography, data collection, data reduction, heavy-atom substructure determination, multiple isomorphous replacement, single- and multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, molecular graphics, structure refinement, structure validation, coordinate deposition, and structure presentation.

This course was supported with funds provided by the National Cancer Institute.
PARTICIPANTS

Coleman, J., Ph.D., Oregon Health & Science University, Portland
DeMare, S., Ph.D., Lund University, Sweden
Falk, S., M.S., Max-Planck Institute of Biochemistry, Germany
Gronberg, C., M.S., University of Copenhagen, Denmark
Harrison, J., Ph.D., Rutgers University, Camden, New Jersey
Hosford, C., Ph.D., Cornell University, Ithaca, New York
Huber, F., Ph.D., California Institute of Technology, Pasadena
Hughes, S., Ph.D., The Scripps Research Institute, San Diego, California
Igumenova, T., Ph.D., Texas A&M University, College Station
Jikova, A., Ph.D., Institute of Organic & Biochemistry, Czech Republic
Larsen, K., Ph.D., Stanford University, California
Mays, S., Ph.D., Emory University, Atlanta, Georgia
Morales-Perez, C., Ph.D., University of Texas Southwestern Medical Center, Dallas
Sanchez, J., Ph.D., University of Virginia, Charlottesville
Santiago Cuellar, J., M.S., University of Geneva, Switzerland
St. John, S., Ph.D., Purdue University, West Lafayette, Indiana

SEMINARS

McPherson, A., University of California, Irvine: Crystallization of macromolecules I and 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.
Caffrey, M., Trinity College, Dublin, Ireland: Crystallizing membrane proteins for structure-function studies using lipidic mesophases.
Sweet, R., Brookhaven National Laboratory, New York: X-ray sources and optics.
Borek, D., University of Texas Southwestern Medical Center, Dallas: X-ray data processing.
Holton, J., University of California, San Francisco: Tips and tricks for improving diffraction.
Furey, W., University of Pittsburgh, Pennsylvania: Patterson group therapy. Isomorphous replacement and anomalous scattering.
Emsley, P., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: Model-building tools in coot. Smith, C., Stanford University, California: Synchrotron data collection and femtosecond crystallography.
Thorn, A., MRC Laboratory of Molecular Biology, Cambridge, United Kingdom: An Introduction to SHELXC/D/E.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Automated structure solution and model building.
Kleywegt, G., European Bioinformatics Institute, Cambridge, United Kingdom: Just because it’s in nature, doesn’t mean it’s true.
Adams, P., University of California, Berkeley: Structure refinement.
Conway, J., University of Pittsburgh, Pennsylvania: Cryoelectron microscopy for probing the structure of protein complexes.
Programming for Biology

October 12–27

INSTRUCTORS
S. Prochnik, DOE–Joint Genome Institute, Walnut Creek, California
S. Robb, Stowers Institute for Medical Research, Kansas City, Missouri

ASSISTANTS
M. Campbell, Cold Spring Harbor Laboratory
J. Mulvaney, Cold Spring Harbor Laboratory
S. Rynearson, University of Utah, Salt Lake City
S. Ahrendt, University of California, Riverside
J. Orkin, Washington University, St. Louis, Missouri

Web-based tools are no longer enough for today’s biologist who needs to access and analyze large data sets from myriad sources in disparate formats. The need to design and program custom analysis pipelines is becoming ever more pressing as new technologies increase the already exponential rate at which biological data is generated. Designed for lab biologists with little or no programming experience, students left this 2-week course with the bioinformatics and scripting skills necessary to exploit this abundance of biological data. The prerequisite for the course was basic knowledge of UNIX; some scripting experience was helpful. Lectures and problem sets from previous years that cover this background material were available online and students could study this material before starting the course.

This course was supported with funds provided by the National Human Genome Research Institute.
PARTICIPANTS

Ashlee, E., Ph.D., Broad Institute, Cambridge, Massachusetts
Chari, S., Ph.D., Princeton University, New Jersey
Colaneri, A., Ph.D., University of North Carolina, Chapel Hill
Cretuoiu, M., Ph.D., Royal Netherlands Institute for Sea Research, The Netherlands
Cronn, R., Ph.D., USDA Forest Services, Corvallis, Oregon
de Miguel Rojas, C., Ph.D., Michigan State University, East Lansing
Guerra Amorim, C.E., Ph.D., Columbia University, New York
Hah, N., Ph.D., The Salk Institute, La Jolla, California
Ingram, W., Ph.D., Geisinger Health Systems, Danville, Pennsylvania
Jochum, L., M.S., Center for Geomicrobiology, Denmark
Karnkowska, A., Ph.D., Charles University, Prague, Czech Republic
Markus, B., M.S., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
Oppenheim, S., Ph.D., American Museum of Natural History, New York
Robertson, H., Ph.D., University College London, United Kingdom
Suryawanshi, H., Ph.D., Rockefeller University, New York
Thomas, R., Ph.D., Walter Reed Army Institute of Research, Silver Spring, Maryland
Wildschutte, J., Ph.D., University of Michigan, Ann Arbor
Winegardner, A., M.S., McGill University, Canada
Wong, S., M.S., Translational Genomics Research Institute, Phoenix, Arizona
Zakrzewski, A., Ph.D., University College London, United Kingdom

SEMINARS

Prochnik, S., Joint Genome Institute, Walnut Creek, California: Unix logins, Perl I: Scripts. Introduction to NGS.
Cain, S., Ontario Institute for Cancer Research, Toronto, Canada: GMOD and JBrowse.
Gogol, G., Stowers Institute for Medical Research, Kansas City, Missouri: Getting started with IGV.
Haas, B., Broad Institute, Cambridge, Massachusetts: Transcript assembly I. Using RNA-Seq for transcript reconstruction analysis.
Korf, I., University of California, Davis: “BLAST.”
Computational and Comparative Genomics

October 28–November 3

INSTRUCTORS
A. Mackey, HemoShear LLC, Charlottesville, Virginia
W. Pearson, University of Virginia, Charlottesville
L. Stubbs, University of Illinois, Urbana

ASSISTANTS
J. Troy, Illinois Informatics Institute, Urbana
L. Mills, University of Minnesota, Minneapolis
C. Seward, University of Illinois, Urbana-Champaign
D.E. Miller, Stowers Institute for Medical Research, Kansas City, Missouri

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; and genome browsers and genome features.

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. The schedule and lectures for the previous year’s course can be found at fasta.bioch.virginia.edu/cshl/.

This course was supported with funds provided by the National Human Genome Research Institute.

PARTICIPANTS

Ahmad, O., Ph.D., McGill University, Montreal, Canada
Bajak, E., Ph.D., Ross University School of Veterinary Medicine, Basseterre, St. Kitts and Nevis, West Indies
Carmona, D., Ph.D., Toronto University, Mississauga, Canada
Choi, Y., Ph.D., Veracyte, Inc., South San Francisco, California
Clawson, H., B.S., University of California Santa Cruz Genomics Institute, Santa Cruz
Contreras, A., Ph.D., ECCPS, Giessen, Germany
De Raedt, T., Ph.D., Harvard Medical School, Boston, Massachusetts
Grasis, J., Ph.D., San Diego State University, California
Ingram, W., Ph.D., Geisinger Health Systems, Danville, Pennsylvania
Isikhuemhen, O., Ph.D., North Carolina A&T State University, Greensboro
Kaminski, L., Ph.D., Albert Einstein College of Medicine, Bronx, New York
Keinath, M., Ph.D., University of Kentucky, Lexington
Rönnrblad, M., Ph.D., Uppsala University, BMC, Sweden
Sabik, O., Ph.D., University of Virginia, Charlottesville
Santiago, J., M.S., Rosalind Franklin University of Medicine, North Chicago, Illinois
Stahlhut, C., Ph.D., Cold Spring Harbor Laboratory
Uchiyama, R., Ph.D., University of Virginia, Charlottesville
Ungerbäck, J., Ph.D., California Institute of Technology, Pasadena
Walsh, M., M.D., Memorial Sloan Kettering Cancer Center, New York
Wilkins, B., M.D./Ph.D., Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania
Wong, M., Ph.D., Cold Spring Harbor Laboratory
Yan, J., Ph.D., Ludwig Institute for Cancer Research, La Jolla, California

SEMINARS

Pearson, W., University of Virginia, Charlottesville, Stubbs, L., University of Illinois, Urbana, and Mackey, A., HemoShear LLC, Charlottesville, Virginia: Introduction and overview.
Pearson, W., University of Virginia, Charlottesville: Protein evolution and sequence similarity searching. Practical sequence similarity searching. Multiple sequence alignment. PSSMs, HMMs, and phenotype prediction.
Taylor, J., Johns Hopkins University, Baltimore, Maryland: Galaxy for high-throughput genome analysis. Galaxy visualization. Probing higher-dimension chromatin structure.
Miller, D.E., Stowers Institute for Medical Research, Kansas City, Missouri: Unix command line.
Stormo, G., Washington University, St. Louis, Missouri: Modeling motifs: Collecting data. From motifs to regulatory networks.
Stubbs, L., University of Illinois, Urbana: Introduction to genome biology. Integrating genomics data sets for biological inference.
Antibody Engineering and Phage Display

November 9–22

INSTRUCTORS  G. Silverman, New York University, New York
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia

CO-INSTRUCTOR  S. Kacir, University of Pennsylvania, Philadelphia

ASSISTANTS  K. Noren, New England Biolabs, Ipswich, Massachusetts
S. Gilgunn, Dublin City University, Ireland
M.A. Pohl, Oncobiologics, Inc., Cranbury, New Jersey

Recent advances in the generation and selection of antibodies from combinatorial libraries allow for the rapid production of antibodies from immune and nonimmune sources. This intensive laboratory/lecture course focused on the construction of combinatorial antibody libraries expressed on the surface of phage and selection of desired antibodies from the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *E. coli* was also covered. Epitopes were selected from peptide libraries and characterized.

This course was supported with funds provided by the National Institute of General Medical Sciences.
PARTICIPANTS

Canessa, C., M.D., Tsinghua University, China
Chen, J., Ph.D., University of Chicago, Illinois
Fazly, A., M.S., Massachusetts Institute of Technology, Cambridge
Guler, R., M.S., Royal Institute of Technology, Sweden
Hincapie, R., Ph.D., Georgia Institute of Technology, Atlanta, Georgia
Jan, Y., Ph.D., EMD Millipore, Germany
Lindbo, S., Ph.D., KTH Royal Institute of Technology, Sweden
Min, J., M.S., Duke University, Durham, North Carolina
Mohamed, S., Ph.D., Cairo University, Egypt
Netles, K., Ph.D., The Scripps Research Institute, San Diego, California
Nikonorova, Y., B.S., State Research Center of Virology, Novosibirsk, Russia
Oliveira, F., Ph.D., Biology Institute, Brasilia, Brazil
Palchaudhuri, R., Ph.D., Harvard University, Cambridge, Massachusetts
Rajagopal, P., Ph.D., University of Washington, Seattle
Wu, Z., Ph.D., Memorial Sloan Kettering Cancer Center, New York

SEMINARS

Siegel, D., University of Pennsylvania, Philadelphia: Pathogenic B cells responses and new therapeutic approaches: From autos to CARS.
Sidhu, S., University of Toronto, Ontario, Canada: Modulation of cell signaling with synthetic proteins.
Rader, C., Scripps Florida, Jupiter: Novel Antibody Engineering & Conjugation Technologies for Cancer Therapy.
Kelly, K., University of Virginia, Charlottesville: Title unknown.
Silverman, G., New York University, New York: B-cell immunobiology and phage display technology.
Boyd, S., Stanford University, California: B-cell repertoire responses to vaccination and infection.
Advanced Sequencing Technologies and Applications

November 10–22

INSTRUCTORS  
E. Mardis, Washington University School of Medicine, St. Louis, Missouri
G. Marth, University of Utah, Salt Lake City
W.R. McCombie, Cold Spring Harbor Laboratory
A. Quinlan, University of Utah, Salt Lake City
M. Schatz, Cold Spring Harbor Laboratory

CO-INSTRUCTORS  
M. Griffith, Washington University School of Medicine, St. Louis, Missouri
O. Griffith, Washington University School of Medicine, St. Louis, Missouri

ASSISTANTS  
J. Gurtowski, Cold Spring Harbor Laboratory
S. Goodwin, Cold Spring Harbor Laboratory
T. Garvin, Cold Spring Harbor Laboratory
M. Kramer, Cold Spring Harbor Laboratory
M. Nattestad, Cold Spring Harbor Laboratory
J. Wasserscheid, McGill University, Montreal, Canada
A. Ward, University of Utah, Salt Lake City
A. Farrell, University of Utah, Salt Lake City
S. McGrath, Washington University School of Medicine, St. Louis, Missouri
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
J. Walker, Washington University School of Medicine, St. Louis, Missouri

Over the last decade, massively parallel DNA sequencing has markedly impacted the practice of modern biology and is being used in the practice of medicine. The constant improvement of
these platforms means that costs and data generation timelines have been reduced by orders of
magnitude, enabling investigators to conceptualize and perform sequencing-based projects that
heretofore were time-, cost-, and sample number–prohibitive. Furthermore, the application of
these technologies to answer questions previously not experimentally approachable is broadening
their impact and application. However, data analysis remains a complex and often vexing chal-
lenge, especially as data volumes increase.

This course was supported with funds provided by the National Human Genome Research
Institute.

PARTICIPANTS

Bennett, J., M.D./Ph.D., University of Washington, Seattle
Chu, S., D.V.M., University of Missouri, Columbia
Coughlin, D., Ph.D., Widener University, Chester,
Pennsylvania
Dupnik, K., M.D., Weill Cornell Medical College,
New York
Ehmsen, J., M.D./Ph.D., Johns Hopkins School of Medicine,
Baltimore, Maryland
Lesiak, A., Ph.D., University of Washington, Seattle
Pengpeng, L., Ph.D., Stanford University, California
Marshall, A., Ph.D., University of Western Ontario, Canada
McNair, C., Ph.D., Thomas Jefferson University,
Philadelphia, Pennsylvania
Preidis, G., Ph.D./M.D., Baylor College of Medicine,
Houston, Texas
Rosello Diez, A., Ph.D., Memorial Sloan Kettering Cancer
Center, New York
Saunders, C., Ph.D., University of Pennsylvania, Philadelphia
Silbereis, J., Ph.D., Yale University, New Haven, Connecticut
Sung, Y.J., Ph.D., Washington University, St. Louis, Missouri
Thodosakis, N., M.D./Ph.D., Yale School of Medicine,
New Haven, Connecticut
Tudor, J., Ph.D., University of Pennsylvania, Philadelphia
Wan, J., M.D./Ph.D., Cancer Research Cambridge Institute,
United Kingdom
Wiesner, T., M.D., Memorial Sloan Kettering Cancer Center,
New York
Xu, L., Ph.D., NVSL, APHIS, USDA, Orient, New York
Zhang, Y., Ph.D., University of Massachusetts Medical
School, Worcester

SEMINARS

Macosko, E., Broad Institute, Cambridge, Massachusetts:
DropSeq technology.
Harkins, T., Swift BioSciences, Inc., Ann Arbor, Michigan:
Enhanced NGS library preparation approaches.
Mardis, E., McDonnell Genome Institute, St. Louis,
Missouri: NGS-based cancer genomics.
Boellmann, F., Illumina, Inc., San Diego, California:
BaseSpace demonstration.

Chapman, B., BC Biomedical, Chiliwach, British Columbia:
BCBIO NGS analysis platform.
Dewar, K., McGill University, Montreal, Canada: Introduction
to genome assembly of PacBio and Illumina data.
Elemento, O., Weill Cornell Medical School, New York:
Introduction to epigenomics analysis.
Scientific Writing Retreat

December 2–6

INSTRUCTORS  
C. Lambert, Cold Spring Harbor Laboratory  
S. Matheson, Cell Press

WRITING COACHES  
L. DeMare, Cold Spring Harbor Press  
S. Gary, Cold Spring Harbor Laboratory  
C. Lambert, Cold Spring Harbor Laboratory  
S. Matheson, Cell Press  
S. Mao, Cell Press  
J. Rubin, Columbia University School of Medicine, New York

The Scientific Writing Retreat was new in 2015 and funded by a National Institutes of Health NIGMS grant for Innovative Programs to Enhance Research Training. The primary goal of the retreat was to have participants progress significantly on writing projects such as manuscripts, grant proposals, and research statements, all while improving the clarity and effectiveness of their professional writing. It included a mix of more traditional invited lectures and less structured blocks of time where students could work individually on their own writing projects. The less structured sessions included small writing groups and feedback sessions as well as one-on-one coaching. The retreat trained 24 students, primarily postdoctoral fellows and junior faculty from all areas of biology. CSHL will continue to offer the writing retreat each autumn through at least 2019.
PARTICIPANTS

Ballou, E., Ph.D., University of Aberdeen, United Kingdom
Balmer, S., Ph.D., Memorial Sloan Kettering Cancer Center, New York
Boucher, J., Ph.D., University of Massachusetts Medical School, Worcester
Brakora, K., Ph.D., University of California, San Francisco
Datta, A., Ph.D., Tulane University, New Orleans, Louisiana
Deshpande, A., Ph.D., University of California, San Francisco
Graham, A., Ph.D., University of Oklahoma Health Sciences Center, Oklahoma City
Guo, Y., Ph.D., Massachusetts Institute of Technology, Cambridge
Hazlewood, R., Ph.D., Vanderbilt Medical Center, Nashville, Tennessee
Hunter, G., Ph.D., University College London, United Kingdom
Kim, Y., Ph.D., Penn State University College of Medicine, Hershey, Pennsylvania
Koehler, I., Ph.D., USDA ARS, Urbana, Illinois
Landry, M., Ph.D., Massachusetts Institute of Technology, Cambridge
Marakalala, M., Ph.D., Harvard School of Public Health, Boston, Massachusetts
Marie, C., Ph.D., University of Virginia, Charlottesville
Memi, F., Ph.D., University of Connecticut Health Center Farmington, Connecticut
Perez, O., M.D., Temple University, Philadelphia, Pennsylvania
Portugal, S., Ph.D., National Institutes of Health, Rockville, Maryland
Reis, T., Ph.D., University of Colorado Medical School, Aurora
Rister, J., Ph.D., New York University, New York
Schaffer, A., Ph.D., University of California, San Diego, La Jolla
Swaminathan, D., Ph.D., University of California, Irvine
Vayttaden, S., Ph.D., NIAID, National Institutes of Health, Bethesda, Maryland
Wang, X., Ph.D., The Jackson Laboratory, Bar Harbor, Maine

SEMINARS

Johnson, L., Alan Alda Center for Communicating Science at Stony Brook University, New York: Improvisation for better communication.
Rubin, J., Columbia University School of Medicine, New York: Grant proposals and fellowships applications.

Lucenko, K., Sharma, S., Alan Alda Center for Communicating Science at Stony Brook University, New York: Reworking abstracts for the lay summaries required by NIH and NSF.
The Genome Access Course

INSTRUCTORS
A. Gordon, New York Genome Center, New York
E. Hodges, Vanderbilt University School of Medicine, Nashville, Tennessee
G. Howell, The Jackson Laboratory, Farmington, Connecticut
B. King, Mount Desert Island Biological Laboratory, Bar Harbor, Maine
J. Ward, Middlebury College, Vermont

LECTURERS
M. Schatz, Cold Spring Harbor Laboratory
M. Hammell, Cold Spring Harbor Laboratory
T. Baslan, Memorial Sloan Kettering Cancer Center, New York

The Genome Access Course (TGAC) is an intensive 2-day introduction to bioinformatics that was held four times in 2015 and trained more than 125 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 2015 included Genome Sequencing and Assembly, the UCSC Genome Browser, Ensembl, Comparative Genome Analysis, Functional Genomic Elements and the ENCODE Project, Gene Set Enrichment and Pathway Analysis, Sequence Polymorphisms, Next-Generation Sequence Data 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.
As in 2013 and 2014, one TGAC in 2015 was held in Manhattan at the New York Genome Center (NYGC, September 2–4). The course was one of several collaborative training projects with the NYGC, for which CSHL is an institutional founding member. It had a very high enrollment of 42 students, ~25% of whom were from local New York institutions. CSHL plans to offer the Manhattan course again in late summer 2016 in collaboration with the New York Genome Center and annually thereafter as long as demand for the course continues.

This course was supported with funds provided by the Helmsley Charitable Trust.

March 30–April 1 (Cold Spring Harbor Laboratory)

Students: 41

PARTICIPANTS

Ahmadi Afzadi, M., Swedish University of Agricultural Sciences, Alnarp
Ahn, S.-J., University of Florida, Gainesville
Allotey, L., Mayo Clinic Rochester, Rochester, Minnesota
Atkinson, E., Northwest College, Powell, Wyoming
Bernitz, J., Ichan School of Medicine at Mount Sinai, New York
Brown, J., Harvard Medical School, Boston, Massachusetts
Chanda, S., Stanford University, California
Coschigano, K., Ohio University, Athens
Dahia, C., Hospital for Special Surgery, New York
Erzurumluoglu, M., University of Bristol, United Kingdom
Feng, W., Memorial Sloan Kettering Cancer Center, New York
Freyer, L., Memorial Sloan Kettering Cancer Center, New York
Ghasemkhani, M., Swedish University of Agricultural Sciences, Alnarp
Giam, L., Stanford University, California
Haldar, S., Case Western Reserve University School of Medicine, Cleveland, Ohio
Hutchins, E., California Institute of Technology, Pasadena
Issa, R., Institute of Genetic Engineering and Biotechnology, Cairo, Illinois
Kluger, M., Yale University School of Medicine, New Haven, Connecticut
Latimer, K., Novartis Institutes for BioMedical Research, Cambridge, Massachusetts
Lizarraga, C., Donald Danforth Plant Science Center, St. Louis, Missouri
Meller, J., Missouri State Health Lab, Jefferson City
Nguyen, J., Mayo Clinic Florida, Jacksonville, Florida
Ning, M., University of Illinois at Chicago
Ono, K., National Institutes of Health, Bethesda, Maryland
Patel, S., Memorial Sloan Kettering Cancer Center/Clarkson University, New York
Seto, J., New York City College of Technology, Brooklyn
Shah, A., Stanford University, California
Shakola, F., St. John's University, Richmond Hill, New York
Shi, Z., University of Notre Dame, Indiana
Sukumaran, S., Monell Chemical Senses Center, Philadelphia, Pennsylvania
Tiriac, H., Cold Spring Harbor Laboratory
Uribe, R., California Institute of Technology, Pasadena
White, A., University of Texas Southwestern Medical Center, Dallas
Wu, D., NIDCD, National Institutes of Health, Bethesda, Maryland
Xiang, S., University of South Florida, Tampa
Yang, H.-S., Brigham and Women's Hospital, Boston, Massachusetts
Yang, L., Princeton University, New Jersey
Yu, M., University of Chicago, Illinois
Zeineddin, M., St. Jude Children's Research Hospital, Memphis, Tennessee
Zhang, X., University of South Florida College of Medicine, Tampa
Zheng, J., University of Bristol, United Kingdom

September 2–4 (New York Genome Center)

Students: 41

PARTICIPANTS

Amador, H.C., Technical University of Denmark, Kongens Lyndy
Baars, O., Princeton University, New Jersey
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Larkins, C., University of Florida, Gainesville
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Mitchell, K., National Cancer Institute, Bethesda, Maryland
Nafa, K., Memorial Sloan Kettering Cancer Center, New York
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Petrova, R., Columbia University, New York
Pettee, K., University of Toledo-Health Science Campus, Ohio
Poi, M., The Ohio State University, Columbus
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Serino, G., Sapienza Università di Roma, Italy
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Yu, X., Rutgers Cancer Institute of New Jersey, New Brunswick
Zingone, A., National Cancer Institute, Bethesda, Maryland

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The Laboratory acknowledges the generosity of the following companies that loaned equipment and reagents to the various courses:

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**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.

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<td>Sculpting neuronal connections: The logic and mechanisms of axon growth and pruning</td>
<td>Anne Churchland</td>
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<td>Tom Misteli, Ph.D., Senior Investigator, Associate Director, Center for Cancer Research, National Cancer Institute, National Institutes of Health</td>
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<td>David Spector</td>
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<td>George Church, Ph.D., Professor of Genetics, Harvard University</td>
<td>Radical recodings of biological systems</td>
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<td>Richard Axel, M.D., Ph.D., Howard Hughes Medical Institute Investigator; Professor, Columbia University</td>
<td>Order from disorder: Internal representations of the olfactory world</td>
<td>Tony Zador</td>
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<td>From Charcot to Lou Gehrig: Gene silencing therapy in ALS and beyond</td>
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<td>Chemical strategies toward developing new drugs</td>
<td>David Tuveson</td>
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<td>Surprising physics of DNA and nucleosomes and potential roles in gene regulation</td>
<td>Justin Block Kinney</td>
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<td>Elaine Ostrander, Ph.D., National Human Genome Research Institute, National Institutes of Health</td>
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<td>Mikala Egeblad</td>
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<td>Linda Van Aelst</td>
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<td>Jennifer Doudna, Ph.D., Howard Hughes Medical Institute Investigator;</td>
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<td>Leemor Joshua-Tor</td>
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<tr>
<td>Professor, Department of Molecular &amp; Cell Biology and Department of</td>
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<td>Chemistry, University of California, Berkeley</td>
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<td>Technology; Member, Whitehead Institute</td>
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<td>Feng Zhang, Ph.D., Assistant Professor, Department of Brain and</td>
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<td>Cognitive Sciences; Core Faculty Member, the Broad Institute,</td>
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<td>Massachusetts Institute of Technology</td>
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<td>Regulation in Infection Biology, Helmholtz Centre For Infection</td>
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<td>Research; affiliated with Mims @ the Department of Molecular Biology</td>
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<td>of Umeå University and Hannover Medical School</td>
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<td>Wei Yang, Ph.D., Section Chief, Laboratory of Molecular Biology,</td>
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<td>Leemor Joshua-Tor</td>
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<td>National Institute of Diabetes and Digestive and Kidney Diseases</td>
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<td>David Kleinfeld, Ph.D., University of California, San Diego</td>
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<td>Florin Albeanu</td>
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<td>Richard Palmiter, Ph.D., Howard Hughes Medical Institute; Professor,</td>
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<td>Bo Li</td>
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<td>Adrian Bracken, Ph.D., Assistant Professor of Medical Molecular</td>
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<td>Alea Mills</td>
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<td>Genetics, Trinity College Dublin</td>
<td>promote cellular quiescence</td>
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<td>How plant immune receptor complexes recognize pathogens and</td>
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<td>The role of low-complexity domains in information transfer from gene</td>
<td>David Spector</td>
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<td>Biochemistry, UT Southwestern Medical Center</td>
<td>to message to protein</td>
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<td>Hans Clevers, M.D., Ph.D., Professor of Molecular Genetics, Director</td>
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<td>David Tuveson</td>
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<td>of Research, Princess Maxima Center for Pediatric Oncology, Hubrecht</td>
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IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor Laboratory's In-House Seminars were initiated to provide a semiformal avenue for communication among the various research groups at the Laboratory. The seminars also afford a necessary opportunity for graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

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<td>Stephen Shea</td>
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<td>Manzar Hossain</td>
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<td>Jen Beshel</td>
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<td>Tomoki Nomakuchi</td>
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BANBURY CENTER
EXECUTIVE DIRECTOR’S REPORT

It was in June 1975 that the Cold Spring Harbor Laboratory Board of Trustees passed a motion accepting “…enthusiastically and with gratitude” Charles Robertson’s gift of his estate. The official opening was in May 1977 when Francis Crick gave a talk on “How Scientists Work,” and the first meeting was held in April 1978. The Center has been extraordinarily successful, fulfilling Robertson’s visions of contributing to science through promoting exchanges of information and ideas. Since 1978, Banbury has held more than 650 meetings attended by more than 13,000 participants coming from 40 countries and every state in the United States with one exception—Alaska. These raw numbers do not convey the influence that Banbury meetings have had, and continue to have, in the development of biomedical research. This influence is felt not only in research but also in areas of science policy. Charles Robertson, I hope, would feel that his gift has been well used.

Funding of the Banbury Center Program

As a preamble to the 2015 report, I want to make some remarks about changes in the Banbury Center program over the years, changes driven primarily by changes in funding. For many years, the generous contributions of companies to the Cold Spring Harbor Laboratory Corporate Sponsor Program (CSP) were a source of stable funding both for the Banbury Center program and for supporting meetings on the main campus. The CSP funds were particularly valuable because
although they were restricted to the support of meetings, they were not restricted to the funding of meetings on specific topics. The choice of which meetings should be supported by Corporate Sponsor Program funds was left to the directors of the two programs.

The Banbury allocation of the CSP funds was invaluable. First, the funds provided support for about one-third of the Banbury program, thus providing a firm foundation for the Center. Second, the CSP funds could be and were used to fund meetings on basic research for which support would otherwise have been difficult to find. This enabled the Center to have meetings on a wide variety of topics.

Membership in the CSP dwindled as the large number of mergers in the pharmaceutical and biotechnology industries reduced the pool of potential members and declined precipitously following the recession. Although matters have improved, several major companies who left in the period 2008–2012 have not returned and the Program remains severely depleted. The consequences of this are visible in the 2015 program, where meetings on cancer and relating to the brain dominate because funding is relatively more available for these topics. Jim Watson and Hakon Heimer have been particularly helpful in finding funds for the former and latter meetings, respectively. And, of course, we are tremendously grateful to our current Corporate Sponsors for their continuing support.

2015 in Numbers

Despite the continuing funding difficulties, the 2015 program proved to be a busy one. The Conference Room was used for 37 events, including 21 Banbury Center meetings. There were 621
participants at these meetings, drawn from 35 states with California, Maryland, Massachusetts, and New York leading the way. The proportion of non-U.S. participants was a little lower this year at 16%, coming from 17 countries. Thirty percent of participants were female. As usual, there were summer lecture courses and the Watson School of Biological Sciences came for two week-long courses. In 2015, a strategic alliance was established between CSHL and the then North Shore–Long Island Jewish healthcare system (now Northwell Health) to promote scientific and clinical research on cancer. The Center is being used for joint meetings between researchers and oncologists, helping to promote collaborations between the two institutions.

HIV/AIDS

Although the Banbury Center has a long history of meetings on HIV/AIDS, the 2015 meeting HIV-1 and How to Kill a Killer: Attempts at Total or Functional Cure of HIV-1 was the first meeting on HIV/AIDS in 26 years. The first meeting was in 1983 when William Topp (CSHL) and Bijan Safai (Memorial Sloan Kettering Cancer Center, New York) organized Acquired Immunodeficiency Syndrome (AIDS) and Human Immunodeficiency Virus (HIV). By 1988, it was known that HIV was the causative agent, but the virus was complex and its interactions with the host cell were hard to unravel. The Banbury meetings in 1988 and 1989 on the Control of HIV Gene Expression dealt with these questions: What were the HIV genes and what did they do in the cell? Perhaps it was thought that the plethora of AIDS/HIV meetings in subsequent years made superfluous Banbury meetings on the topic, but a new development led to the 2015 meeting. In 2014 two children given antiretroviral therapy and believed to be HIV-free relapsed when the therapy stopped. The question this raised was where might the virus have been hiding? The organizers, Robert Gallo (University of Maryland, Baltimore), Steven Deeks (University of California, San Francisco), and Robert Siliciano (Johns Hopkins University, Baltimore, Maryland) and the participants reviewed topics such as the mechanism of HIV latency, how to better assay HIV, and what leads to the reversal of HIV latency.

Neurodegenerative Disorders

Neurodegenerative disorders characterized by protein misfolding have long been a focus of Banbury Center meetings. The first meeting on Alzheimer’s disease was held in 1982, and 33 years later, we continued to review the research on the basic mechanism of abnormal protein folding and aggregation in Biophysical Properties and Biological Significance of Amyloid-β Assemblies.

Stanley Prusiner, who won the Nobel Prize for Physiology or Medicine, contributed a paper on prions to the 1982 meeting and subsequently organized a meeting on prions, the first of a series, the latest of which, Therapeutic Approaches to Prion Disease and Other Neurodegenerative Conditions, was held in 2015. It was interesting to see how the field has progressed from studies on the nature and mechanism of prion formation through to enabling discussion of possible therapies.

Psychiatric Disorders

This general field continues to be a mainstay of the Banbury program, and through the work of Hakon Heimer, we were able to hold four meetings this year focused on therapeutic approaches to relieving the distress of mental illness. Therapeutic Use of Ketamine for Treating Severe Depression: Risks and Potential followed up on a meeting on ketamine and depression held in 2012. That meeting reviewed what is known of ketamine’s mode of action and its effectiveness in relieving profound depression. The 2015 meeting examined the impediments to the use of ketamine for treating depression. It was a very effective meeting, and it may lead to new treatment recommendations.
Deep brain stimulation (DBS) has been used successfully to treat people with severe mental illness, although it is not clear exactly how it brings benefit to patients. However, DBS requires implantation of electrodes in the brain—surgery that severely limits the application of DBS. Participants in *Brain Rhythms as Potential Targets for Intervention in Cognitive Dysfunctions* reviewed the current state of methods to stimulate the brain noninvasively, electrically or magnetically. In addition to scientific and clinical considerations involved, the meeting closed with a session discussing the ethical and regulatory issues.

Schizophrenia is a devastating disorder, both for the individual and for the family. The lives of many people with schizophrenia could be radically improved if they had full access to proven treatments and support services. These include pharmacologic therapy, cognitive training, and environmental support. However, it has been difficult to identify which treatments are effective and which can be widely deployed. This is especially difficult because of considerable variation in individual responses—one individual may experience relief by a treatment that is ineffective in others. Participants in *Thriving with Schizophrenia* evaluated the research base underlying treatment/support methods and identified those that are promising but require further research.

The fourth of the meetings was on the genetics and neurobiology of borderline personality disorder (BPD), a disabling condition with high morbidity and substantial mortality. Indeed, 10% of people with BPD die by suicide, and BPD is a risk factor for treatment-refractory depression. A high proportion of psychiatrically hospitalized patients carry the diagnosis of BPD, and even the best available treatment typically does not attenuate all of the symptoms of BPD, even in those who respond well to treatment. Although neglected for many years, research is revealing that it is a highly heritable disorder with associated functional abnormalities in brain circuitry. Nevertheless, an understanding of its pathophysiology remains elusive. The aim of this conference was to draw attention to the neurobiological research in BPD and to bring together individuals from a variety of disciplines to drive forward scientific knowledge that will advance treatment for this disorder.

**Cancer**

Although studies of the genetics and genomics of cancer continue apace, there has been a significant increase in research on the metabolic changes in cancer cells. This is of particular interest to Jim Watson, whose Oliver Grace Cancer Fund provided support for two meetings in this field. The first, *Mitochondria and Cancer*, was organized by Navdeep Chandel (Northwestern University, Chicago, Illinois) and David Sabatini (Whitehead Institute, Cambridge, Massachusetts) and reviewed what is known in the field. Although the majority of cancer cells display functional mitochondria, there are small subsets of cancer cells with impaired mitochondrial function. These cells can nevertheless perform biosynthetic functions for macromolecule synthesis. Overall, the accumulating evidence now suggests that mitochondrial bioenergetics, biosynthesis, and signaling are required for tumorigenesis. One goal of the meeting was to identify possible targets of mitochondrial metabolism for cancer therapy.

The second meeting had a similar goal but considered the problem more broadly. *Tumor Cell Metabolism: Finding New Targets for Therapeutic Intervention* was organized by Lewis Cantley (Weill Cornell Medical College, New York) and Steven McKnight (University of Texas Southwestern Medical Center, Dallas). Participants reviewed new metabolic targets, discussed biomarkers which may predict which tumors are likely to respond to drugs that hit these targets, examined potential...
mechanisms of resistance to such therapies, and discussed drug combinations that could prevent resistance.

**Promoting Research**

Banbury takes special pride in meetings that have contributed to research by helping organizations and foundations plan future research or by training the next generation of scientists. The Boehringer Ingelheim Fonds once again came to Banbury for their Fellows Retreat, in which the fellows are given training in writing and giving talks. This is the 10th year that the Foundation has brought its fellows to Banbury and we hope very much that we will continue to help the Foundation fulfill its goal of training the next generation of scientists.

Banbury also hosted a meeting that was designed to help a new foundation develop its plan of action. The Foundation is HeritX and the goals of the meeting were succinctly encapsulated in its title: *Preventing Inherited BRCA Cancer: A Think Tank for Innovative Strategies, Milestone Objectives, and Research Priorities*. Over two days of intensive review and discussion, HeritX developed a road map for its future.

**Acknowledgments**

Banbury works well because of the hard work of many people, not least Janice Tozzo and Pat Iannotti in the Banbury Center office. Now, after 7 years of ensuring that I completed tasks on time and did not forget meetings, Janice has retired to concentrate on her glass sculptures and Michelle Corbeaux has come to take her place. Basia Polakowski continues to welcome and look after participants in Robertson House, and Jose Covera, Joe McCoy, and Saul Covera keep the estate looking beautiful, coping with huge quantities of leaves in the fall and snow in the winter. Culinary Services, Facilities, and the Meetings Office play key roles in the operation of the Center. The meetings would not be the success they are without the contributions of organizers and participants, the generosity of the Laboratory’s Corporate Sponsors and the other donors who fund our meetings, and the Laboratory’s scientists who continue to support the Center.

Jan A. Witkowski

*Executive Director*
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<td>Creating Patient-Specific Neural Cells for the In Vitro Study of Human Disorders</td>
<td>F. Gage, R. Jaenisch</td>
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<td>Beyond the Wheat Genome</td>
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<td>T. Insel, J. Chung</td>
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<td>Thriving with Schizophrenia</td>
<td>L. Dixon, H. Heimer, J. Kane, M. Munetz, R. Heinssen</td>
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<td>Integrated Translational Science Center Workshop</td>
<td>L. Baker, L. Ellis, E. Liu, A. Schott, D. Tuveson</td>
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<td>September 1–4</td>
<td>Mitochondria and Cancer</td>
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<td>Therapeutic Approaches to Prion Disease and Other Neurodegenerative Conditions Associated with Protein Misfolding</td>
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<td>September 27–30</td>
<td>Therapeutic Developments for ALS: Antisense, Gene Therapy, and Stem Cells</td>
<td>L. Bruijn, T. Miller, C. Svendsen, D. Sah</td>
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<td>October 13–16</td>
<td>HIV-1 and How to Kill a Killer: Attempts at Total or Functional Cure of HIV-1</td>
<td>R. Gallo, S. Deeks, R. Siliciano</td>
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<td>October 18–20</td>
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<td>November 11–13</td>
<td>Preventing Inherited BRCA Cancer: A Think Tank for Innovative Strategies, Milestone Objectives, and Research Priorities</td>
<td>A. Ashworth, T. Bock, L. Brody</td>
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<td>November 15–18</td>
<td>How Can the Genetics and Neurobiology of Borderline Personality Disorder Contribute to Its Diagnosis and Treatment?</td>
<td>J. Oldham, A. New</td>
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<td>December 7–10</td>
<td>Tumor Cell Metabolism: Finding New Targets for Therapeutic Intervention</td>
<td>L. Cantley, S. McKnight</td>
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The Boehringer Ingelheim Fonds’ international program of support for Ph.D. fellowships 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 2015 stay at Banbury was the eighth occasion that 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.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Opening Remarks and All About BIF—Part 1: C. Walther, Boehringer Ingelheim Foundation, Mainz, Germany

Communication—Why and How?: K. Achenbach, Boehringer Ingelheim Foundation, Mainz, Germany

N. LeBrasseur, DNA Medical Communications, New York: Writing techniques and how to structure papers: Writing assignment 1.

B. Tansey, Vanderbilt University, Nashville, Tennessee: Preparing and delivering a scientific talk.
Group A: 4-min PowerPoint presentations, videotaped with replay and feedback.

N. LeBrasseur, DNA Medical Communications, New York: Discussion of writing assignment 1: Writing assignment 2.
B. Tansey, Vanderbilt University, Nashville, Tennessee: PowerPoint presentations.
N. LeBrasseur, DNA Medical Communications, New York: Return and discussion of writing assignment 2.
B. Tansey, Vanderbilt University, Nashville, Tennessee: Group B: 3-min PowerPoint presentations, videotaped with replay and feedback.

M. Skobe, Mount Sinai School of Medicine: Career talk.
K. Ris-Vicari, Katie Ris-Vicari Graphic Design, Bethpage, New York, and
M. Hansen, Nature Publishing Group, New York: How to design figures.

All About BIF—Part 2 and Feedback: C. Walther, Boehringer Ingelheim Foundation, Mainz, Germany

Guided Walking Tour on CSHL Campus
Exercise is regarded as a key component of healthy living, and yet there appears to be little consensus on how exercise regimens can be used most efficiently and optimized for promoting health. Participants in this meeting considered several questions on health and exercise, including the following: What conditions benefit from exercise? What mediates the beneficial effects of exercise? What exercise regimens are effective? What, if any, are the interactions between exercise and nutrition? Can understanding the pathways by which exercise brings about its effects be useful in guiding the development of drugs?

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction: R. Pate, University of South Carolina, Columbia

SESSION 1: Type-2 Diabetes and the Metabolic Syndrome
Chairperson: W. Kraus, Duke University Medical Center, Durham, North Carolina
J. Wojtaszewski, University of Copenhagen, Denmark: Mechanisms in exercise-induced muscle insulin sensitivity.
B. Goodpaster, Sanford–Burnham Medical Research Institute, Orlando, Florida: The impact of exercise on type-2 diabetes and cardiometabolic risk.
A. Kriska, University of Pittsburgh, Pennsylvania: Impact of the successful diabetes prevention program’s lifestyle intervention. What progress has been made in efforts to translate this knowledge to the public health arena?

General Discussion

SESSION 2: Healthy Aging
Chairperson: R. Fielding, Tufts University, Boston, Massachusetts
A. Wagers, Harvard University, Cambridge, Massachusetts: Local and systemic regulators of aging phenotypes in mammalian tissue.
T. Church, Pennington Biomedical Research Center, Baton Rouge, Louisiana: Exercise and quality of life: The forgotten benefit.
D. Buchner, University of Illinois, Champaign: Public health strategies for promoting physical activity and healthy aging.

SESSION 3: Muscle Hypertrophy and Sarcopenia
Chairperson: B. Goodpaster, Sanford–Burnham Medical Research Institute, Orlando
T. Hornberger, University of Wisconsin, Madison: The potential role of lysosomal targeting in the mechanical activation of mTOR.
R. Fielding, Tufts University, Boston, Massachusetts: Muscle hypertrophy and sarcopenia: Clinical science.

General Discussion

SESSION 4: Selected Presentations
Chairperson: B. Goodpaster, Sanford–Burnham Medical Research Institute, Orlando, Florida
J. Mitchell, University of Pennsylvania, Philadelphia: The benefits of physical activity on bone density in childhood are dependent on genetic variation at known bone density loci.
J. Valentine, University of Texas Health Science Center, San Antonio: Inhibition of NF-kB causes muscle weakness and severe exercise intolerance.
X. Wang, University of South Carolina, Columbia: Changes in nonexercise activity thermogenesis with participation in exercise training in older women: Preliminary results from the WeWalk study.

SESSION 5: Physical Activity and Cognition
Chairperson: J. Pivarnik, Michigan State University, East Lansing
J. Reilly, University of Strathclyde, Glasgow, United Kingdom: Physical activity and cognition: Clinical science.
R. Pate, University of South Carolina, Columbia: Physical activity and children’s learning; Ready for public health prime time?

General Discussion

SESSION 6: Parental Exercise/Exercise during Pregnancy and Child Health
Chairperson: J. Reilly, University of Strathclyde, Glasgow, United Kingdom
L. Goodyear, Joslin Diabetes Center and Harvard Medical School, Cambridge, Massachusetts: Effects of maternal exercise on metabolic health of offspring.

SESSION 7: Exercise Resistance: Genetic Nonresponse and Compliance with Exercise
Chairperson: D. Buchner, University of Illinois, Champaign
C. Bouchard, Pennington Biomedical Research Center, Baton Rouge, Louisiana: Poor response of cardiorespiratory fitness with exposure to regular exercise: Evidence for a genetic basis.
B. Kraus, Duke University Medical Center, Durham, North Carolina: Molecular predictors of exercise nonresponsiveness and program adherence.
R. Dishman, University of Georgia, Athens: Exercise adherence and compliance: Motivation and genes.

SESSION 8: Concluding Discussion: Health and Exercise
Moderator: L. Goodyear, Joslin Diabetes Center and Harvard Medical School, Cambridge, Massachusetts

- Are all patterns of exercise equally effective in promoting health?
- How little exercise is needed to achieve a benefit?
- What can be done for public health?
Optimal cognitive and emotional processing arises from the simultaneous and successive interplay of large ensembles of neurons in multiple brain regions. One organizing principle appears to be the temporal dynamics of systems-level neural activity, such as electrophysiologically recorded oscillations, including their coordination across frequency bands and with action potentials. The goals of this Banbury meeting included examining current knowledge about how systems-level temporal dynamics supports cognitive and emotional processing and how this might be used to enhance cognitive and emotional processing.

There is also the potential to improve functional outcomes in patients with neuropsychiatric disorders. In addition, participants looked more broadly at the ethical and regulatory considerations for electrophysiological treatments.

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Goals and Vision for the Meeting: B. Osborn, National Institute of Mental Health, Rockville, Maryland

SESSION 1: Temporal Dynamics for Therapeutic Use

Chairperson: R. Cohen Kadosh, University of Oxford, United Kingdom

T. Buschman, Princeton University, New Jersey: Dynamic synchronous ensembles for creating flexible task representations.
B. Pesaran, New York University, New York: Controlling computations in large-scale neural circuits: Today and tomorrow.

B. Postle, University of Wisconsin, Madison: Concurrent TMS unmask functions relevant endogenous components of task-related EEG.

G. Thut, University of Glasgow, Scotland: Modulating brain oscillations by transcranial brain stimulation to drive brain function.


C. Herrmann, Oldenburg University, Germany: Modulating brain oscillations and cognitive functions with transcranial alternating current stimulation (tACS).

Y. Saalmann, University of Wisconsin, Madison: Thalamocortical dynamics in cognition.

B. Voytek, University of California, San Diego: Dynamic network communication as a unifying neural basis for cognition, development, aging, and disease.

D. Tucker, University of Oregon, Eugene: Spatial and temporal resolution of geodesic transcranial electrical neuromodulation.

V. Sohal, University of California, San Francisco: Rescuing PFC-dependent cognition by restoring interneuron-driven gamma oscillations.

F. Frohlich, University of North Carolina, Chapel Hill: Rational design of brain stimulation that targets oscillation dynamics.

A. Fenton, New York University, New York: The microstructure of cognition-associated neural coordination can distinguish between cognitive states and identify dysfunction.

General Discussion

SESSION 2: Clinical Implications

Chairperson: A. Pascual-Leone, Harvard Medical School, Boston, Massachusetts

E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois: Excitation-inhibition coordination.

A. Benasich, Rutgers University, Newark, New Jersey: Defining the functional role of cortical oscillatory dynamics across maturation and identifying potential biomarkers as targets for noninvasive behavioral interventions.


S. Molholm, Albert Einstein College of Medicine, Bronx, New York: Sensorimotor networks and multisensory networks. In autism as well as other DDs, these domains are clearly impacted and have implications for the clinical phenotype.

S. Loo, University of California, Los Angeles: Rescuing PFC-dependent cognition by restoring interneuron-driven gamma oscillations.

R. Cohen Kadosh, University of Oxford, United Kingdom: Improving learning outcomes in participants with typical and atypical development using transcranial random noise stimulation.

General Discussion

SESSION 3: Ethical and Regulatory Considerations

Chairperson: B. Postle, University of Wisconsin, Madison

M. Barilan, Tel Aviv University, Israel: Moral enhancement: Is it enhancement? Is it moral?

P. Reiner, University of British Columbia, Vancouver, Canada: Neuroethics of optimizing brain rhythms.

E. Civillico, Food and Drug Administration, Silver Springs, Maryland: Regulatory science and brain oscillations.

A. Pascual-Leone, Harvard Medical School, Boston, Massachusetts: Report from the Institute of Medicine meeting.

M. Garvey, National Institute of Mental Health, Rockville, Maryland: Translational and clinical steps forward.

SESSION 4: Future Directions

Chairperson: B. Postle, University of Wisconsin, Madison: Where are we now?

• Gap areas and opportunities.
• Setting the agenda for future research.
• Documenting meeting outcomes.
There is general agreement that self-assembly of the amyloid-β (Aβ) peptide in brain tissue leads to neurodegeneration in Alzheimer’s disease (AD), and it is also clear that many different self-assembled states exist both in vitro and in vivo. However, there is no consensus on which of these self-assembled states has the most significant role in AD development and how these lead to neurodegeneration. Participants critically reviewed what is currently known of the molecular structures of Aβ fibrils and oligomers, the biological effects of self-assembled Aβ, methods for detecting Aβ assemblies in human and transgenic animal brain tissue, and approaches to inhibiting clinically significant Aβ assemblies.

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Opening Remarks and Overviews: K. Ashe, University of Minnesota Medical School, Minneapolis
R. Tycko, National Institute of Diabetes, Digestive, and Kidney Disease, Bethesda, Maryland
SESSION 1
Chairperson: K. Ashe, University of Minnesota Medical School, Minneapolis
S. Lesné, University of Minnesota, Minneapolis: Breaking the biological code of amyloid-β oligomers.
D. Eisenberg, University of California, Los Angeles: Structure of the toxic core of α-synuclein amyloid, the protein associated with the development of Parkinson’s disease.
D. Walsh, Harvard University, Boston, Massachusetts: Amyloid-β and beyond: Studies using human brain, cell lines, and recombinant peptides.

SESSION 2
Chairperson: J. Kelly, Scripps Research Institute, La Jolla, California
R. Tycko, National Institutes of Health, Bethesda, Maryland: Molecular structures and structural variations in amyloid-β fibrils.
Y. Ishii, University of Illinois, Chicago: In vitro and in vivo structures of Aβ(1-42) fibrils and spherical oligomers.
T. Härd, Swedish University of Agricultural Sciences, Uppsala, Sweden: Solid-state NMR and molecular modeling provide structural information on amyloid-β protofibrils.

SESSION 3
Chairperson: R. Tycko, National Institutes of Health, Bethesda, Maryland
J. Kelly, Scripps Research Institute, La Jolla, California: Toward a structure-proteotoxicity relationship in the transhyretin amyloidoses.

SESSION 4
Chairperson: D. Eisenberg, University of California, Los Angeles
K. Ashe, University of Minnesota Medical School, Minneapolis: Temporal, spatial, and structural relationships of type-1 and type-2 amyloid-β oligomers.
T. Knowles, University of Cambridge, United Kingdom: Kinetics of protein aggregation.
D. Knopman, Mayo Clinic, Rochester, Minnesota: The detection of suspected non-Alzheimer pathophysiology in cognitively normal persons and implications for the pathogenesis of Alzheimer’s disease.

SESSION 5
Chairperson: R. Nussinov, National Institutes of Health, Leidos Biomedical Research, Frederick, Maryland
J. Nowick, University of California, Irvine: X-ray crystallographic structures of oligomers of peptides derived from amyloid-β.
C. Soto, University of Texas Medical School, Houston: Detection of amyloid-β oligomers in human CSF and blood through amplification of seeding.
B. Ma, National Institutes of Health, Leidos Biomedical Research, Frederick, Maryland: The known and unknown structural aspects of amyloid-β peptide globular oligomers.


Review and Summary
Creating Patient-Specific Neural Cells for the In Vitro Study of Brain Disorders

April 14–17

FUNDED BY Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY F. Gage, Salk Institute for Biological Studies, San Diego, California
R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

Most of the studies of human brain and neuronal function in phenotypically normal and neurological/psychiatric patients have been performed using noninvasive imaging methods that do not give single-cell resolution or performed on postmortem tissues often representing the end-stage of life and disease. The recent advances in reprogramming somatic cells, including the production of induced pluripotent stem cells and induced neuronal phenotypes, have changed the experimental landscape and opened new possibilities. However, a number of pressing issues need to be resolved if this strategy is to become standard for clinically relevant modeling of neurological/psychiatric diseases. For example, more and better protocols are needed for differentiating patient-specific neural cells into specific subtypes. Participants in this Banbury meeting examined some of these issues, including the advantages and disadvantages of the various techniques being used to generate neural cells and how to obtain disease-relevant subtypes of neurons.

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Opening Remarks and Overviews: F. Gage, Salk Institute for Biological Studies, San Diego, California
R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
SESSION 1: Epigenetics

J. Wu, Salk Institute for Biological Studies, La Jolla, California: A molecular and cellular toolbox for studying brain disorders.
N. Benvenisty, Hebrew University of Jerusalem, Givat Ram, Jerusalem: Modeling epigenetic disorders using human pluripotent stem cells.
R. McKay, Lieber Institute for Brain Development, Baltimore, Maryland: Using the dynamic variation between pluripotent stem cells to define the biology of individual human genomes.

SESSION 2: New Strategies

D. Panchision, National Institute of Mental Health, Bethesda, Maryland: Next steps in NIMH support for reprogrammed cell research.
O. Bruestle, University of Bonn, Germany: Toward industrialization of stem-cell-based disease modeling and drug development.
A. Ebert, Medical College of Wisconsin, Milwaukee: Methods for generating more purified astrocyte cultures from iPSCs.
P. Vanderhaeghen, University of Brussels, Belgium: From pluripotent stem cells to cortical circuits.
M. Lancaster, Austrian Academy of Science, Vienna, Austria: Using cerebral organoids to examine pathogenesis of neurodevelopmental disorders.
F. Vaccarino, Yale University, New Haven, Connecticut: Telencephalic organoids model early developmental trajectories in autism.

SESSION 3: Modeling Developmental/Psychiatric Diseases

K. Brennand, Mount Sinai School of Medicine, New York: Modeling predisposition to schizophrenia using hiPSCs.
C. Marchetto, Salk Institute for Biological Studies, La Jolla, California: Modeling human complex neurological disorders using neural cells.
E. Morrow, Brown University, Providence, Rhode Island: Live cell imaging of neurodevelopment in cells from patients with Christianson syndrome.
A. Sawa, Johns Hopkins University School of Medicine, Baltimore, Maryland: Multifaceted clinical study in psychiatry that utilizes patient stem cells.
H. Song, Johns Hopkins University School of Medicine, Baltimore, Maryland: Patient-derived iPSC modeling of major psychiatric disorders.
A. Muotri, University of California, San Diego: Clearance of endogenous L1 retroelements in the cytosol by TREX1 prevents neuronal toxicity.

SESSION 4: Modeling Neurological Diseases

R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts, and F. Soldner, Whitehead Institute, MIT, Cambridge, Massachusetts: In vitro modeling of complex neurological disease.
R. Livesey, Gurdon Institute, Cambridge, United Kingdom: Insights into mechanisms of Alzheimer’s disease pathogenesis from human stem cell models.
M. McConnell, University of Virginia School of Medicine, Charlottesville: hiPSC-based neurogenesis to study brain mosaicism.
A. Kaykas, Novartis Institute for BioMedical Research, Cambridge, Massachusetts: A pipeline to identify phenotypes in hPSC-derived neurons.
L. Studer, Memorial Sloan Kettering Cancer Center, New York: Modeling neural development and disease in human pluripotent stem cells.
S. Temple, Neural Stem Cell Institute, Rensselaer, New York: Using iPSCs to model age-related macular degeneration.

Review and Summary
Neuronal Response Variability and Correlation

April 19–22

FUNDED BY The Swartz Foundation

ARRANGED BY L. Abbott, Columbia University, New York
K. Rajan, Princeton University, New Jersey
J. Reynolds, Salk Institute for Biological Studies, La Jolla, California

This meeting brought together experimentalists and theorists seeking to understand neuronal response variability and its implications for cortical computation: Is variability “noise” or is it a signature of important computations that we have yet to understand? Participants examined related questions such as how these response fluctuations emerge and how they are modulated by cognitive state, such as attentional state. Variability and correlations are extremely important probes into the workings of neural circuits, but what is the precise relationship between the state and dynamics of a neural circuit and the variability and correlations observed in experiments?

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
SESSION 1
D. Angelaki, Baylor College of Medicine, Houston, Texas: How can single sensory neurons predict perception?
S. Fusi, Columbia University School of Medicine, New York: High dimensional neural representations.
B. Doiron, University of Pittsburgh, Pennsylvania: Network mechanisms for the control of noise correlations in recurrent cortical populations.
M. Murthy, Princeton Neuroscience Institute, New Jersey: Rapid sensorimotor integration and song variability in *Drosophila*.

SESSION 2
M. Fee, Massachusetts Institute of Technology, Cambridge: Dedicated circuits for the generation and shaping of neuronal variability underlying vocal learning in the songbird.
A. Fairhall, University of Washington, Seattle: Context-dependent modulation of variability through a basal ganglia circuit.
M. Crair, Yale University School of Medicine, New Haven, Connecticut: Do activity correlations drive circuit development?

SESSION 3
K. Harris, University College London, United Kingdom: Coupling of single neurons to populations in sensory cortex.
A. Nandy, Salk Institute for Biological Studies, La Jolla, California: Optogenetically induced low-frequency correlations impair perception.
A. Kohn, Albert Einstein College of Medicine, Bronx, New York: A role for coordinated neuronal activity in corticocortical signaling.
B. Cumming, National Eye Institute, National Institutes of Health, Bethesda, Maryland: Correlated noise that reflects psychophysical task instructions.

SESSION 4
C. Bargmann, The Rockefeller University, New York: Variation and circuit states in probabilistic behaviors.
J. Freeman, HHMI, Janelia Farm Research Campus, Ashburn, Virginia: Measuring and manipulating neural computation.

SESSION 5
T. Engel, Stanford University, California: Modulation of cortical state by selective visual attention.
A. Thiele, Newcastle University, Newcastle upon Tyne, United Kingdom: Efficient decoding in the face of response variability.
B. Hansen, Salk Institute, La Jolla, California: Neural mechanisms underlying attention-related changes in brain state.
A. Mitra, Washington University School of Medicine, St. Louis, Missouri: The restless brain: How intrinsic activity organizes brain function.
Beyond the Wheat Genome

April 25–27

FUNDED BY The Genome Analysis Centre, Norwich, United Kingdom

ARRANGED BY M. Caccamo, The Genome Analysis Centre, Norwich, United Kingdom

In 2014, the wheat genome project reached a significant milestone with the publication of the first whole-genome reference assembly, although this reference still requires much work. This group convened to examine both the tasks that remain and whether forming an Expert Working Group would promote the completion of those tasks. It was decided that such a group would be useful and plans were laid to develop an Expert Working Group.

Opening Remarks and Introduction: M. Caccamo, The Genome Analysis Centre, Norwich, United Kingdom

SESSION 1

N. Stein, Leibniz Institute of Plant Genetics and Crop Plants, Gaterslaben, Germany: Barley genome.
D. Edwards, University of Western Australia, Crawley, and C. Pozniak, University of Saskatchewan, Saskatoon, Canada: Wheat chromosome assembly.
E. Akhunov, Kansas State University, Manhattan: Wheat natural diversity.
K. Krasileva, The Genome Analysis Centre, Norwich, United Kingdom and C. Uauy, John Innes Centre, Norwich, United Kingdom: Wheat tillering resources.
A. Hall, University of Liverpool, Liverpool, United Kingdom and M. Bevan, John Innes Centre, Norwich, United Kingdom: Wheat epigenetics.

SESSION 2: Breakout Groups: Topics for Discussion

A. Innovative strategies for improving the genome sequence.
B. Resequencing wheat genomes.
C. Functional genomics resources.
D. Creating a gene expression atlas.
E. Epigenetic analyses.
F. Databases and open access data standards.

K. Mayer, Helmholtz Zentrum München, Neuherberg, Germany: Annotation resources.
P. Kersey, European Bioinformatics Institute, Cambridge, United Kingdom, and D. Ware, Cold Spring Harbor Laboratory: Bioinformatics resources.
SESSION 3: Reports from Breakout Groups

Group 1
M. Caccamo, The Genome Analysis Centre, Norwich, United Kingdom
K. Mayer, Helmholtz Zentrum München, Neuherberg, Germany
A. Hall, University of Liverpool, United Kingdom
C. Pozniak, University of Saskatchewan, Saskatoon, Canada

Group 2
E. Akhunov, Kansas State University, Manhattan
K. Krasileva, The Genome Analysis Centre, Norwich, United Kingdom
M. Bevan, John Innes Centre, Norwich, United Kingdom
J. Batley, University of Western Australia, Crawley
N. Hall, University of Liverpool, United Kingdom
P. Kersey, European Bioinformatics Institute, Cambridge, United Kingdom

Group 3
R. McCombie, Cold Spring Harbor Laboratory
M. Clark, The Genome Analysis Centre, Norwich, United Kingdom
C. Uauy, John Innes Centre, Norwich, United Kingdom
D. Edwards, University of Western Australia, Crawley
S. Sukumaran, CIMMYT, El Batán, Mexico
D. Ware, Cold Spring Harbor Laboratory

Discussion and Next Steps

C. Pozniak, D. Edwards
Cold Spring Harbor Laboratory is renowned worldwide for its education programs, from high school level to the highest professional levels. One of the Banbury Center’s contributions is to host the NIMH-sponsored “Brain Camp.” The goal of the Brain Camp is to identify areas of neuroscience that are of interest and relevance to psychiatrists and to communicate these to a small group of outstanding psychiatry residents and research fellows. Some of the most distinguished and thoughtful neuroscientists in the country came as guest speakers to the meeting. The goal of the series of meetings is to develop a neuroscience curriculum that can eventually be shared with psychiatry training programs around the country.

SESSION 1

T. Insel, National Institute of Mental Health, Bethesda, Maryland: Will psychiatry become clinical neuroscience?

C. Liston, Weill Cornell Medical College, New York: Mechanisms of prefrontal cortical circuit dysfunction in chronic stress and depression.

SESSION 2

S. Amara, National Institute of Mental Health, Bethesda, Maryland: Neurotransmitter transporters: A few curious
observations, many interesting collaborations…and a little advice.


J. LeDoux, New York University, New York: Coming to terms with fear and anxiety.

T. Jovanovic, Emory University School of Medicine, Atlanta, Georgia: The brain on trauma: Neurobiological correlates of trauma exposure in an urban population.

Roundtable Discussion on Teaching Neuroscience to Psychiatrists

D. Ross, Yale University, New Haven, Connecticut

E. Rosemond, National Institute of Mental Health, Bethesda, Maryland: NIMH funding for M.D. and M.D.-Ph.D.s.

SESSION 3

S. Hollingsworth Lisanby, Duke University School of Medicine, Durham, North Carolina: Space, time, and context: The “when,” “where,” and “how” of focal neuromodulation in psychiatry.

C. Zarate, National Institute of Mental Health, Bethesda, Maryland: Developing rapid acting antidepressants: Major hurdles, current progress, and future strategies.
Thriving with Schizophrenia

June 14–17

FUNDED BY The Margaret Clark Morgan Foundation

ARRANGED BY L. Dixon, Columbia University, New York
H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island
J. Kane, North Shore LIJ Health System, Glen Oaks, New York
M. Munetz, Northeast Ohio Medical University, Rootstown, Ohio
R. Heinssen, National Institute of Mental Health, Bethesda, Maryland

The lives of many people with schizophrenia could be radically improved if they had full access to proven treatments and support services. There have been strong calls for full implementation of the Mental Health Parity Act passed by the U.S. Congress in 2008, especially in the context of the Affordable Care Act of 2010. However, it has been difficult to identify which treatments are effective and which can be widely deployed. The participants in this meeting reviewed and critically evaluated current therapies and support programs for schizophrenia that can or may improve quality of life. Among the topics discussed were pharmacologic therapy, rehabilitation through cognitive training and environmental support, holistic healthcare, and social aspects of therapy.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Workshop Introduction: H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island

SESSION 1: Assessing Knowledge about Treatment and Support for People with Schizophrenia

Chairperson: H. Heimer, Schizophrenia Research Forum, Providence, Rhode Island
L. Dixon, Columbia University, New York: The PORT process: Overview and psychosocial treatments.

Discussants Present Key Points
D. Addington, University of Calgary, Canada
J. Kane, North Shore-LIJ Health System, Glen Oaks, New York
L. Davidson, Yale University, New Haven, Connecticut

SESSION 2: Focus on Topical Issues: What Is the Evidence?
Chairperson: M. Munetz, Northeast Ohio Medical University, Rootstown, Ohio
Issue 1: Medication: Balancing Good and Harm
J. Kane, Hofstra North Shore-LIJ School of Medicine, Glen Oaks, New York

Issue 2: Cognitive Behavioral Therapy: For What and for Whom?
T. Wykes, Institute of Psychiatry, London, United Kingdom
P. McKenna, University of Barcelona, Spain

Issue 3: Supportive Services: Peer Networks, Housing, Employment
M. Chinman, Rand Corporation, Pittsburgh, Pennsylvania
L. Davidson, Yale University, New Haven, Connecticut

Issue 4: First-Episode Research: Lessons for Schizophrenia
J. Kane, North Shore-LIJ Health System, Glen Oaks, New York
D. Addington, University of Calgary, Canada

SESSION 3: Real-World Laboratories
Chairperson: J. Kane, North Shore-LIJ Health System, Glen Oaks, New York

SESSION 4: Barriers to Dissemination and Implementation: What Do We Want to Achieve?
Chairperson: R. Heinssen, National Institute of Mental Health, Bethesda, Maryland

Short Presentations
L. Sederer, Columbia University, New York
L. Rosenberg, National Council for Behavioral Health, Washington, DC
A. Sperling, National Alliance on Mental Illness, Arlington, Virginia

Discussants
K. Myrick, Substance Abuse and Mental Health Services Administration, Rockville, Maryland
L. Herman, Northeast Ohio Medical University, Rootstown, Ohio
F. Frese, Northeast Ohio Medical University, Hudson, Ohio

SESSION 5: Getting to Where We Need to Be
Chairpersons: J. Kane, North Shore-LIJ Health System, Glen Oaks, New York, and R. Heinssen, National Institute of Mental Health, Bethesda, Maryland

F. Frese
T. Wykes, L. Sederer
Integrated Translational Science Center Workshop

June 18–20

FUNDED BY National Cancer Institute and The Hope Foundation

ARRANGED BY L. Baker, University of Michigan, Ann Arbor
L. Ellis, University of Texas, Houston
E. Liu, The Jackson Laboratory, Bar Harbor, Maine
A. Schott, University of Michigan, Ann Arbor
D. Tuveson, Cold Spring Harbor Laboratory

The Integrated Translational Science Center (ITSC) Workshop included clinician scientists from SWOG and basic scientists from Cold Spring Harbor Laboratory (CSHL) and the Jackson Laboratory (JAX). Its goal was to foster interactions between science in the clinic and at the bench. This was an interactive workshop with talks, posters, laboratory demonstrations, and brainstorming sessions to generate ideas for collaborative projects.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Core Strengths

D. Tuveson, Cold Spring Harbor Laboratory: Cold Spring Harbor Laboratory: Core strengths.
E. Liu, The Jackson Laboratory, Bar Harbor, Maine: Jackson Laboratory: Core strengths.
L. Baker, University of Michigan, Ann Arbor: Workshop Overview.

A. Schott
POSTER SESSION

SESSION 2: Lectures by Scientists
D. Tuveson, Cold Spring Harbor Laboratory: Tissue models/organoids.
J. Hicks, Cold Spring Harbor Laboratory: Single-cell analysis.
E. Liu, The Jackson Laboratory, Bar Harbor, Maine: JAX animal models, PDX mouse, and genomics technologies.

POSTER SESSION

SESSION 3: Demonstrations of the Techniques Discussed
Organoids
L. Baker, Cold Spring Harbor Laboratory
D. Engle, Cold Spring Harbor Laboratory

D. Ohlund, Cold Spring Harbor Laboratory

Single-Cell Analysis
J. Hicks, Cold Spring Harbor Laboratory
J. Alexander, Cold Spring Harbor Laboratory
J. Kendall, Cold Spring Harbor Laboratory

JAX Platforms (All): Humanized Mice, PDX, Genomics Platforms
J. Keck, The Jackson Laboratory
A. Cheng, The Jackson Laboratory
P. Robson, The Jackson Laboratory

SESSION 4: CSHL Campus Tour

SESSION 5: ITSC Pilot Submission Process: How to Write a Successful Proposal
Cancer cells rely on mitochondrial metabolism to provide the necessary building blocks for macromolecule (nucleotides, lipids, amino acids) synthesis as well as ATP and NADPH essential for cell proliferation. Multiple substrates feed mitochondrial metabolism, including pyruvate and glutamine. As a consequence of oxidative metabolism, the mitochondria of cancer cells produce significant amounts of ROS to activate proximal signaling pathways and promote tumorigenesis. Although the majority of cancer cells display functional mitochondria, there are small subsets of cancer cells with impaired mitochondrial function. Despite the inability to generate mitochondrial ATP, these cancer cells demonstrate remarkable metabolic plasticity, allowing them to conduct biosynthetic functions for macromolecule synthesis. Overall, the accumulating evidence now suggests that mitochondrial bioenergetics, biosynthesis, and signaling are required for tumorigenesis. Thus, emerging studies have begun to unveil the targeting of mitochondrial metabolism as a promising avenue for cancer therapy.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction and Goals of Workshop: N. Chandel, Northwestern University, Chicago, Illinois
SESSION 1: Mitochondrial Metabolism of Cancer Cells In Vivo
Chairperson: E. Gottlieb, Cancer Research UK Beatson Institute, Glasgow, United Kingdom
T. Fan, University of Kentucky, Lexington: Human lung cancer metabolome, from bench to bedside.
M. VanderHeiden, Massachusetts Institute of Technology, Cambridge: Role of respiration in cancer cell proliferation.

SESSION 2: Mitochondria and Metabolic Stress
Chairperson: J. Rutter, University of Utah School of Medicine, Salt Lake City
E. White, Rutgers University, New Brunswick, New Jersey: Mitochondrial quality control and cancer.

SESSION 3: Mitochondrial Metabolites and Cancer
Chairperson: E. White, Rutgers University, New Brunswick, New Jersey
Marcia Haigis, Harvard Medical School, Boston, Massachusetts: PHD3 and fat metabolism.
E. Gottlieb, Cancer Research UK Beatson Institute, Glasgow, United Kingdom: Metabolic adaptations and liabilities of TCA cycle-truncated tumors.

SESSION 4: Mitochondria and Aging
Chairperson: E. White, Rutgers University, New Brunswick, New Jersey
J. Auwerx, Ecole Polytechnique Federale de Lausanne, Switzerland: Mitonuclear communication in metabolism and aging.
A. Brunet, Stanford University, California: Metabolic and epigenetic regulation of aging.

SESSION 5: Biology of Complex I
Chairperson: K. Salnikow, National Cancer Institute, NIH, Rockville Maryland
D. Sabatini, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Systematic approaches to study metabolism.
M. Kaeberlein, University of Washington, Seattle: Identification of interventions that delay mitochondrial disease in Complex-I-deficient mice.

SESSION 6: Targeting Mitochondrial Electron Transport Chain
Chairperson: K. Salnikow, National Cancer Institute, NIH, Rockville, Maryland.
L. Trotman, Cold Spring Harbor Laboratory: Mitochondria and prostate metastasis.
G. Draetta, MD Anderson Cancer Center, Houston, Texas: OXPHOS inhibitors as cancer therapeutics.
D. Sykes, Massachusetts General Hospital, Boston: Targeting DHODH and endogenous uridine biosynthesis in the treatment of patients with acute myeloid leukemia.

**SESSION 7: Regulators of Mitochondrial Metabolism**

Chairperson: E. White, Rutgers University, New Brunswick, New Jersey
J. Rutter, University of Utah School of Medicine, Salt Lake City: The impact of pyruvate metabolism on stemness in normal and cancer settings.
C. Metallo, University of California, San Diego, La Jolla: Mitochondria and amino acid metabolism.

**SESSION 8: Mitochondria Regulation of Adaptive Immunity**

Chairperson: M. Haigis, Harvard Medical School, Boston, Massachusetts
P. Ashton-Rickardt, Imperial College London, United Kingdom: The protein LEM promotes CD8+ T-cell immunity through effects on mitochondrial respiration.
J. Powell, Johns Hopkins University, Baltimore, Maryland: Dissecting and exploiting metabolism in T cells.

**General Discussion: M. VanderHeiden**, Massachusetts Institute of Technology, Cambridge

**SESSION 9: Mitochondria and ATM-p53 Pathway**

Chairperson: N. Chandel, Northwestern University, Chicago, Illinois
M. Kastan, Duke Cancer Institute, Durham, North Carolina: ATM: Bridging DNA damage responses and metabolic regulation.
P. Hwang, National Heart, Lung & Blood Institute, Bethesda, Maryland: Targeting mitochondria for cancer prevention in Li-Fraumeni syndrome.
R. Sordella, Cold Spring Harbor Laboratory: p53′ evil twin linked to tissue injury and spread of cancer.
A. Yang, Boston Biomedical Inc., Cambridge, Massachusetts: BBI-608 targets cancer stem cells and prevents tumor relapse and metastasis.

**Meeting Summary: N. Chandel**, Northwestern University, Chicago, Illinois
Therapeutic Approaches to Prion Disease and Other Neurodegenerative Conditions Associated with Protein Misfolding

September 15–18

FUNDED BY Cold Spring Harbor Laboratory Corporate Sponsor Program

ARRANGED BY J. Collinge, University College London, United Kingdom
J. Kelly, The Scripps Research Institute, La Jolla, California

Neurodegenerative diseases such as Alzheimer's disease arguably represent the greatest challenge to healthcare systems in developed countries with aging populations, and as yet no effective disease-modifying therapies are available. Major advances in understanding prion disease, a rare but high-profile cause of dementia, are now leading to the development of therapeutics. In parallel, it is becoming clear that similar molecular processes of protein misfolding and aggregation (“prion-like mechanisms”) are involved in the much commoner dementias such as Alzheimer's disease. Participants in this meeting examined progress in development of therapeutics for prion diseases and other potentially tractable protein misfolding disorders and considered their wider relevance for diseases of major public health and economic importance.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Meeting Introduction: J. Collinge, University College London, United Kingdom
J. Kelly, The Scripps Research Institute, La Jolla, California

Introduction to Prion-Like Mechanisms and Relevance to Human Neurodegenerative Disease: J. Collinge, University College London, United Kingdom

SESSION 1: Small-Molecule Therapeutics for Prion Disease
Chairperson: J. Collinge, University College London, United Kingdom
B. Caughey, NIAID Rocky Mountain Laboratories, Hamilton, Montana: Practical detection of prions and prion inhibitors.
M. Farrow, University College London, United Kingdom: Development of a clinical candidate drug for treatment of prion disease.
G. Legname, Scuola Internazionale Superiore di Studi Avanzati, Trieste, Italy: Tackling prion diseases with multitarget and theranostic small molecules.
K. Kuwata, Gifu University, Japan: Toward a first in human trial of a medical chaperone for prion disease.

A. Giese, Ludwig-Maximilians-University, Munich, Germany: Targeting toxic oligomers with small molecules: Chances and challenges.

General Discussion: Key Issues in Developing Small-Molecule Therapeutics

SESSION 2: Immunotherapeutic Approaches

Chairperson: F. Tagliavini, Instituto Neurologico Carlo Besta, Milan, Italy

C. Glabe, University of California, Irvine: Anti-amyloid antibodies: What do they see that we don’t?

J. Collinge, University College London, United Kingdom: Passive immunotherapy of prion disease.

T. Wisniewski, New York University School of Medicine, New York: Vaccination approaches for prion and Alzheimer’s disease.

M. Horiuchi, Hokkaido University, Sapporo, Japan: Immun- and cell therapy as possible treatments for prion disease.

General Discussion: Key Issues in Immunotherapeutic Approaches

SESSION 3: Alzheimer’s and Tauopathies

Chairperson: D. Walsh, Brigham and Women’s Hospital, Boston, Massachusetts

D. Walsh, Brigham and Women’s Hospital, Boston, Massachusetts: The activity and forms of Aβ in mild AD brains.

A. Nicoll, UCL Institute of Neurology, London, United Kingdom: Dissecting the PrP:Aβ interaction.

S. Strittmatter, Yale University School of Medicine, New Haven, Connecticut: Cellular prion protein signal transduction complex mediating amyloid-β oligomer synaptotoxicity in Alzheimer’s disease.

M. Hutton, Eli Lilly and Company Ltd., Surrey, United Kingdom: Tau pathology: Critical Tau species required for propagation and spreading.

F. Tagliavini, Instituto Neurologico Carlo Besta, Milan, Italy: Novel approach to Alzheimer’s disease therapeutics based on a natural variant of A-β that hinders amyloidogenesis.

S. Olson, University of California Institute for Neurodegenerative Diseases, San Francisco: A high-content assay approach to drug discovery for neurodegenerative disorders.

General Discussion: Key Issues in Alzheimer’s and Tauopathies

SESSION 4: Proteostasis, Protein Misfolding, and Therapeutic Approaches to Neurodegenerative Diseases

Chairperson: J. Kelly, The Scripps Research Institute, La Jolla, California

A. Horwich, Yale University, New Haven, Connecticut: Studies of mice with SOD-1-linked ALS.


J. Kelly, The Scripps Research Institute, La Jolla, California: Adapting proteostasis to ameliorate degenerative diseases.

Closing Remarks: J. Collinge, University College London, United Kingdom, and J. Kelly, The Scripps Research Institute, La Jolla, California
Therapeutic Use of Ketamine for Treating Severe Depression:
Risks and Potential

September 20–22

FUNDED BY Cold Spring Harbor Corporate Sponsor Program; Alkermes, Inc.; and Janssen Research & Development

ARRANGED BY R. Robinson Beale, Blue Cross of Idaho, Meridian
H. Heimer, Cold Spring Harbor Laboratory
J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Ketamine is purported to be the only truly new and effective therapy discovered for depression in the past 50 years. Individuals who are severely depressed and often suicidal respond rapidly to the common anesthetic, reporting dramatic mood changes within minutes. The benefits can last for several weeks, giving other standard therapies an opportunity to take effect. Despite this, ketamine is not widely available. This meeting convened representatives from academia, government, and private payers, as well as patient advocates, to discuss the risks and possibilities presented by broader use of ketamine for treating severe depression.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Meeting Introduction: H. Heimer, Cold Spring Harbor Laboratory

H. Heimer, A. Malhotra
SESSION 1: Current Knowledge and Ongoing Research
Chairperson: S. Mathew, Baylor College of Medicine, Houston, Texas
D. Charney, Icahn School of Medicine, New York, and M. Fava, Massachusetts General Hospital, Boston: Ketamine in depression: Clinical trial evidence.
G. Sanacora, Yale University, New Haven, Connecticut: Lessons from other proposed rapidly acting antidepressants: Pharmacological specificity and nonspecific clinical effects.
E. Ehrich, Alkermes, plc, Waltham, Massachusetts: Opioid modulation as a treatment of major depressive disorder.
C. Nemeroff, University of Miami, Florida: Meta-analysis of ketamine and related compounds in depression.

Where Next for Research?
A. Schatzberg, Stanford University, California
M. Hillefors, National Institute of Mental Health, Bethesda, Maryland

SESSION 2: Current Clinical Practice and Challenges
Chairperson: R. Robinson Beale, Blue Cross of Idaho, Meridian, Idaho
A. Dahan, Leiden University Medical Center, Leiden, The Netherlands: Ketamine pharmacokinetics and pharmacodynamics: Efficacy and toxicity.

SESSION 3: Stakeholder Concerns
Chairperson: A. Malhotra, North Shore-LIJ Health System, Glen Oaks, New York
M. Isaac, European Medicines Agency, London, United Kingdom
D. Hartman, Ketamine Advocacy Network, Seattle, Washington
P. Summergrad, Tufts Medical Center, Boston, Massachusetts
R. Robinson Beale, Blue Cross of Idaho, Meridian, Idaho
I. Wiechers, Office of Mental Health Operations, Department of Veterans Affairs, West Haven, Connecticut

SESSION 4: Recommendations and Guidelines
Chairperson: H. Goldman, University of Maryland, Potomac
A. Revisiting Key Issues.
B. Discussion of Next Steps.
Therapeutic Developments for ALS: Antisense, Gene Therapy, and Stem Cells

September 27–30

FUNDED BY The Greater New York Chapter of The ALS Association

ARRANGED BY L. Bruijn, The ALS Association, Washington, D.C.
T. Miller, Washington University, St. Louis, Missouri
C. Svendsen, Cedars-Sinai, Los Angeles, California
D. Sah, Voyager Therapeutics, Cambridge, Massachusetts

This workshop discussed the key common challenges in therapeutic development for ALS shared by antisense oligonucleotide, gene therapy, and stem cell modalities, with the goal of identifying steps that will facilitate solutions to these challenges and ultimately enhance the probability of successful clinical development. In particular, translation of delivery, biomarker development, trial design, and regulatory issues was highlighted. These challenges are especially relevant this year with the important progress anticipated in bringing antisense therapy closer to the clinic for SOD1 and C9orf72, and in advancing stem cell therapies already in clinical trials or planned for later this year. Clinicians, scientists, and regulatory experts provided overviews of the current status of the various antisense oligonucleotide, gene therapy, and stem cell programs, setting the stage for discussions around the key common challenges: translation of delivery, biomarker development, trial design, and regulatory issues.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Overview and Workshop Goals: L. Bruijn, ALS Association, Washington, D.C.
SESSION 1: Antisense Oligonucleotide Therapy

Chairperson: T. Miller, Washington University, St. Louis, Missouri
T. Miller, Washington University, St. Louis, Missouri: SOD1.
D. Cleveland, University of California, San Diego: C9orf72.
D. Rodman, miRagen Therapeutics, Boulder, Colorado: microRNA targeting to the CNS.

General Discussion, Highlighting Key Points

SESSION 2: Gene Therapy

Chairperson: R. Bartus, RTBioconsultants, Inc., San Diego, California
K. Bankiewicz, University of California, San Francisco
B. Kaspar, Ohio State University, Columbus
D. Sah, Voyager Therapeutics, Cambridge, Massachusetts

General Discussion Highlighting Key Points

SESSION 3: Stem Cells

Chairperson: D. Rowitch, University of California, San Francisco
J. Glass, Emory University School of Medicine, Atlanta, Georgia: Neuralstem.
C. Svendsen, Cedars-Sinai Medical Center, Los Angeles, California: CIRM/Cedars-Sinai combined stem and gene therapy trial.
N. Maragakis, Johns Hopkins University School of Medicine, Baltimore, Maryland, and J. Campanelli, Q Therapeutics, Inc., Salt Lake City, Utah: Q therapeutics trial.

General Discussion Highlighting Key Points

SESSION 4: Delivery and Biomarker Development

Chairperson: J. Bulte, Johns Hopkins University School of Medicine, Baltimore, Maryland
G. Stewart, Voyager Therapeutics, Cambridge, Massachusetts: Lost in (therapy) translation: It won't work, if it doesn't get there.
N. Boulis, Emory University, Atlanta, Georgia, and C. Svendsen, Cedars-Sinai Medical Center, Los Angeles, California: Stem cell tracking techniques and delivery.
E. Ahrens, University of California, San Diego, La Jolla: Emerging MRI methods to assess cell engraftment and host response.

General Discussion Highlighting Key Points

SESSION 5: Regulatory and Clinical Trial Design Panel Discussion

Chairperson: B. Ravina, Voyager Therapeutics, Cambridge, Massachusetts
Panelists
J. Lebkowski, Asterias Biotherapeutics, Portola Valley, California
J. Berry, Massachusetts General Hospital, Boston
T. Ferguson, Biogen, Cambridge, Massachusetts

General Discussion Highlighting Key Points

SESSION 6: Animal Models

Chairperson: J. Rothstein, Johns Hopkins University School of Medicine, Baltimore, Maryland
R. Baloh, Cedars-Sinai Medical Center, Los Angeles, California: Overview of mouse models.
J. Coates, University of Missouri, Columbia: Canine degenerative myelopathy: A potential disease model of ALS.
A. Burghes, Ohio State University, Columbus: SMA pig model.
Z. Xu, University of Massachusetts Medical School of Medicine, Worcester: Modeling and treatment of sporadic ALS.

General Discussion Highlighting Key Points

Closing Remarks
Disorders of the brain are widespread and impose a tremendous cost on individuals and society. Despite the enormous toll, we are at a near standstill in the development of new treatments for these conditions. Recent technical developments, including optogenetics and chemogenetics, have allowed scientists to identify specific brain circuit elements in both physiological and pathological behavior. Chemogenetics, a method for manipulating cellular function using drug-like compounds and engineered proteins, has shown promise for probing circuit function and has the potential to bridge the gap between genetic analysis of circuit function and treatment. The goal of this meeting was to bring together experts with broad expertise in neurobiology, tool development, primate biology, neurosurgery, and imaging to identify a clear path forward for the use of chemogenetic tools for treatment of human disease.

Introduction: G. Aston-Jones, Rutgers University, Piscataway, New Jersey

SESSION 1: Designing New Chemogenetic Tools
Chairperson: G. Aston-Jones, Rutgers University, Piscataway, New Jersey

J. English, University of North Carolina, Chapel Hill: DREADD 2.0: Novel methods for DREADD production and implementation.
SESSION 2: New Chemogenetic Approaches with Potential Clinical Applications

Chairperson: E. Vazey, University of Massachusetts, Amherst

Y. Hurd, Icahn School of Medicine at Mount Sinai, New York: DREAMM for in vivo pathway-specific brain activity mapping.

S. Dymecki, Harvard Medical School, Boston, Massachusetts: Conditional DREADD knock-in alleles: Lessons from mice.

R. Adan, Utrecht University, The Netherlands: Targeting specific neurons implicated in obesity.

B. Roth, University of North Carolina, Chapel Hill: Chemical biology of chemogenetic actuators.

General Discussion

SESSION 3: Using Chemogenetics to Probe Neuronal Circuit Function and Dysfunction

Chairperson: K. Grant, Oregon National Primate Research Center, Beaverton

G. Aston-Jones, Rutgers University, Piscataway, New Jersey: Dopamine DREADDs, drug seeking, and demand.

S. Ferguson, University of Washington, Seattle: Using DREADDs to map addiction circuits.

B. Li, Cold Spring Harbor Laboratory: Chemogenetic inhibition of the amygdala circuits during fear processing in mice.

SESSION 4: Optimizing Chemogenetic Approaches in Primates

Chairperson: T. Kash, University of North Carolina, Chapel Hill

K. Grant, Oregon National Primate Research Center, Beaverton: Using DREADDs to address functional neurocircuitry and behavioral outcomes in monkeys.

N. Kalin, University of Wisconsin, Madison: Developing DREADDs methodology in nonhuman primates to reversibly manipulate the neural circuitry underlying anxiety disorders.

T. Minamimoto, National Institute of Radiological Sciences, Chiba, Japan: PET imaging of DREADDs in monkeys.

B. Richmond, National Institute of Mental Health, Bethesda, Maryland: The possibilities and problems in controlling cortical connections using DREADDs.

General Discussion

SESSION 5: Making the Leap to Therapy

Chairperson: N. Kalin, University of Wisconsin, Madison

D. Kullmann, University College London, United Kingdom: Chemogenetics for epilepsy: How far from clinical translation?

C. Felder, Neuroscience, Eli Lilly & Co., Indianapolis, Indiana: Recent challenges and advances in GPCR drug discovery: Importance of academic-industrial partnerships.

D. Goldman, National Institute on Alcohol Abuse & Alcoholism, Rockville, Maryland: Chemogenetics for addictions: Challenges and opportunities.

General Discussion and Summary
HIV-1 and How to Kill a Killer: Attempts at Total or Functional Cure of HIV-1

October 13–16

FUNDED BY amfAR; The Foundation for AIDS Research; Gilead Sciences, Inc.; and Office of AIDS Research, NIH, NIDA, and NIAID Division of AIDS

ARRANGED BY R. Gallo, IHV at University of Maryland School of Medicine, Baltimore
S. Deeks, University of California, San Francisco
R. Siliciano, Johns Hopkins University, Baltimore, Maryland

AIDS led to an extraordinary research effort during the past 30 years, and although much has been learned about the basic biology of HIV and therapies have been developed to combat AIDS, there remain unanswered questions. Two are key in our efforts to develop treatments: Can we obtain a complete virological cure? And, if not, can we obtain a functional cure (no further therapy needed)? A select group of top experts and opinion leaders in the field of HIV pathogenesis came to Banbury to critically examine what has been done and what might be done to answer these questions. Previous Banbury Center meetings on HIV and AIDS (1983, 1988, 1989, 1992) were important and timely, and the 2015 meeting proved to be equally so.

Introduction: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Opening Remarks and Questions for the Field: R. Gallo, IHV at University of Maryland School of Medicine, Baltimore

R. Gallo
Measuring the Size of the Latent Reservoir: A Key to Successful Eradication Strategies: R. Siliciano, Johns Hopkins University, Baltimore, Maryland

SESSION 1: Virology

Chairperson: D. Kuritzkes, Brigham & Women’s Hospital, Cambridge, Massachusetts

J. Coffin, Tufts University, Boston, Massachusetts: Intracellular RNA expression in treated and untreated HIV-infected individuals.

J. Mellors, University of Pittsburgh, Pennsylvania: Clonal expansion of HIV-1 reservoirs.

J. Mullins, University of Washington, Seattle: Propagation and comprehensive analysis of individual lineages of HIV-infected cells persisting during ART.

J.V. Garcia-Martinez, University of North Carolina, Chapel Hill: Systemic distribution of the latent HIV reservoir.

E. Verdin, University of California, San Francisco: Molecular mechanisms of HIV latency.

SESSION 2: Immunology

Chairperson: P. Sato, NIH Office of AIDS Research, Bethesda, Maryland

R.-P. Sekaly, Case Western Reserve University, Cleveland, Ohio: Restoring immune homeostasis to eradicate HIV.


W. Greene, Gladstone Institute of Virology and Immunology, San Francisco, California: Death by friendly fire: How HIV turns our innate immune defenses against us.

J. Ananworanich, US Military HIV Research Program, Bethesda, Maryland: Reservoirs and immune activation in blood and tissue compartments from the RV254 Thai cohort.

SESSION 3: Biomarkers

Chairperson: D. Finzi, National Institutes of Health, Bethesda, Maryland

D. Richman, University of California, San Diego: Improving assays to measure the latent reservoir.

D. Kuritzkes, Brigham & Women’s Hospital, Cambridge, Massachusetts: The challenge of identifying surrogate markers for HIV cure.

SESSION 4: Novel Therapies

Chairperson: W. Greene, Gladstone Institute of Virology and Immunology, San Francisco, California

J. Lifson, National Cancer Institute, Frederick, Maryland: The role of NHP models in HIV cure research.


A. Garzino-Demo, University of Maryland, Baltimore: Letting sleeping dogs lie: Targeting T-cell activation.

M. Stevenson, University of Miami, Florida: Strategies other than “purge and kill” to limit viral reservoirs.

SESSION 5: Latency Reversal

Chairperson: D. Margolis, University of North Carolina, Chapel Hill

D. Hazuda, Merck Research Labs, West Point, Pennsylvania: Understanding the activity of HDACIs in vitro and in vivo.

General Discussion Reviewing Key Issues

C. Dieffenbach, E. Verdin
J. Karn, Case Western Reserve University, Cleveland, Ohio: Distinct mechanisms of hormonal control of HIV latency in T cells and microglial cells.

B. Peterlin, University of California, San Francisco: Mechanistic approaches to HIV cure: PKC agonists and latency reversing agents.

F. Romerio, University of Maryland, Baltimore: The HIV-1 antisense transcript AST is an inducer of viral latency.

General Discussion Reviewing Key Issues

SESSION 6: Experimental Medicine and Clinical Trials

Chairperson: S. Read, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

R. Geleziunas, Gilead Sciences, Inc., Foster City, California: TLR7 agonists for HIV.

D. Margolis, University of North Carolina, Chapel Hill: Understanding latency reversal and reservoir clearance.

S. Deeks, University of California, San Francisco: Closing comments: Challenges in translation.

SESSION 7: Commentary

R. Gallo, IHV at University of Maryland, Baltimore

S. Deeks, University of California, San Francisco

R. Siliciano, Johns Hopkins University, Baltimore, Maryland

C. Dieffenbach, National Institute of Allergy and Infectious Diseases, Rockville, Maryland

R. Johnston, amfAR, New York

General Discussion and Summary
The Lustgarten Foundation Scientific Meeting

October 18–20

FUNDED BY
The Lustgarten Foundation

ARRANGED BY
C. Ardito-Abraham, The Lustgarten Foundation, Bethpage, New York
D. Tuveson, Cold Spring Harbor Laboratory

The Banbury Center is always pleased to provide a venue for the Lustgarten Foundation to hold its annual Pancreatic Cancer Scientific Conference. The occasion provides an opportunity for researchers funded by the Foundation to get together and exchange information and ideas, thus enabling the Foundation scientific advisory board to assess progress in the field and plan for the future.

Welcome and Introductions

Research Investigators Progress Reports
S. Leach, Memorial Sloan Kettering Cancer Center, New York
S. Lowe, Memorial Sloan Kettering Cancer Center, New York
C. Fuchs, Dana Farber Cancer Institute, Boston, Massachusetts
M. Muzumdar, Dana Farber Cancer Institute, Boston, Massachusetts
F. McCormick, University of California, San Francisco
T. Jacks, Massachusetts Institute of Technology, Cambridge
S. Fesik, Vanderbilt University School of Medicine, Nashville, Tennessee
C. Der, University of North Carolina, Chapel Hill
G. Verdin, Harvard University, Cambridge, Massachusetts
L. Cantley, Weill Cornell Medical College, New York
T. Hunter, Salk Institute for Biological Studies, La Jolla, California
J. Sage, Stanford University Medical Center, California
D. Simeone, University of Michigan, Ann Arbor
A. Klein, Johns Hopkins University School of Medicine, Baltimore, Maryland
H. Ploegh, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

SAB/KRas Discussion
C. Fuchs, Dana Farber Cancer Institute, Boston, Massachusetts
F. McCormick, University of California, San Francisco

Research Biopsy Study
B. Wolpin, Dana Farber Cancer Institute, Boston, Massachusetts

Distinguished Scholar Progress Reports
D. Tuveson, Cold Spring Harbor Laboratory
R. Evans, Salk Institute for Biological Studies, La Jolla, California
D. Fearon, Cold Spring Harbor Laboratory
B. Vogelstein, Johns Hopkins University, Baltimore, Maryland

C. Ardito-Abraham

R. Evans, J. Watson

B. Vogelstein, L. Cantley
Epilepsy is a common and often devastating neurological disease affecting at least one in 26 Americans at some point in their lives. Critically, more than 30% continue to have seizures despite the many available therapies. Unfortunately, drug development in epilepsy has largely stalled, but epilepsy genetics affords an opportunity to develop new targeted treatments in epilepsy. Participants in this meeting examined progress in the genetics of the epilepsies; the advent of animal and in vitro models allowing the development of medications tailored to genetically defined subtypes of epilepsy; and how to evaluate the efficacy of experimental treatments in cost-effective clinical trials.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Epilepsy Genetics I: Gene Discovery Update

Chairperson: D. Goldstein, Columbia University, New York

S. Berkovic, Austin Health, University of Melbourne, Heidelberg, Australia: Success in genetics and challenges ahead.
E. Heinzen Cox, Columbia University, New York: Gene discovery in severe sporadic epilepsies.

S. Berkovic, University of California, San Francisco; Ron and Sanne Higgins
Epilepsy Fund; Columbia University Institute for Genomic Medicine; Pairnomix; and Clarus Ventures

ARRANGED BY

S. Berkovic, Austin Health, University of Melbourne, Heidelberg, Australia
E. Heinzen Cox, Columbia University, New York
D. Goldstein, Columbia University, New York
D. Lowenstein, University of California, San Francisco
SESSION 2: Functioning Modeling: Etiology, Excitability Networks and Mechanisms

Chairperson: S. Petrou, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia

E. Cooper, Baylor College of Medicine, Houston, Texas: Genetics and structure-function implications of Kv7 channels in epilepsy.

J. Kearney, Northwestern University, Chicago, Illinois: Comparing human vs. mouse sodium channelopathies.

A. Bassuk, University of Iowa, Iowa City: Precision genetics in multiple species.

C. Gross, Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio: microRNA-mediated regulation of potassium channel complexes in epilepsy.

M. Weston, Baylor College of Medicine, Houston, Texas: Interneuronal communication in mTOR ‘opathies’.

E. Rossignol, CHU Ste-Justine Research Center, Montreal, Canada: Developmental interneuronopathy and epilepsy.

SESSION 3: Platforms in Functional Modeling

Chairperson: J. Parent, University of Michigan, Ann Arbor

K. Staley, Massachusetts General Hospital, Boston: Organotypic cultures for drug screens.

W. Frankel, Jackson Laboratory, Bar Harbor, Maine: Recent progress in modeling epilepsy in laboratory mice.


A. Cohen, Harvard University, Cambridge, Massachusetts: All-optical electrophysiology for neuronal disease modeling and drug discovery.

J. Parent, University of Michigan, Ann Arbor: iPSCs in the study of epileptic encephalopathies.

D. Goldstein, Columbia University, New York: Multielectrode arrays for modeling epilepsy mutations.

General Discussion Reviewing Key Issues

SESSION 4: Developing Effective Epilepsy Therapies from Precision Genetics

Chairperson: S. Berkovic, Austin Health, University of Melbourne, Heidelberg, Australia

S. Baraban, University of California, San Francisco: SCN1A: Drug screening in Dravet syndrome zebrafish.

S. Petrou, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia: SCN1A: Modulation of SCN1A by spider toxin.


D. Dlugos, Children’s Hospital of Pennsylvania, Philadelphia: KCNT1: Quinidine in KCNT1 epilepsies.

S. Mullen, Florey Institute of Neuroscience and Mental Health, Melbourne, Australia: KCNT1: A clinical trial for nocturnal frontal lobe epilepsy due to KCNT1 mutation.

I. Scheffer, Florey Institute and University of Melbourne, Australia: Targeted treatments of PCDH19 related epilepsies and mTORopathies.
S. Traynelis, Emory University School of Medicine, Atlanta, Georgia: GRIN2A: GRIN2A mutation and early-onset epileptic encephalopathy: Personalized therapy with memantine.

Group Discussion: Reviewing Key Issues

SESSION 5: Clinical, Social, and Policy Considerations

Chairperson: D. Lowenstein, University of California, San Francisco

A. Poduri, Boston Children's Hospital, Massachusetts: Precision medicine in the epilepsy clinic: Lessons from the early days.


V. Whittemore, National Institute of Neurological Disorders and Stroke, Bethesda, Maryland: Epilepsy within NIH precision medicine initiative.

General Discussion and Summary
Preventing Inherited BRCA Cancer: A Think Tank for Innovative Strategies, Milestone Objectives, and Research Priorities

November 11–13

FUNDED BY HeritX

ARRANGED BY A. Ashworth, University of California Cancer Center, San Francisco
T. Bock, HeritX, Chester, New Jersey
L. Brody, National Human Genome Research Institute, NIH, Bethesda, Maryland

Twenty years ago, the discovery of the BRCA genes was heralded as the most exciting story in medical science. At Banbury in 1995, scientists optimistically predicted that this new genetic information would lead to new treatments within a decade. Yet while much knowledge has been gained about the clinical consequences and individual risk associated with BRCA mutations, the medical management of BRCA carriers is still limited. Families affected by a BRCA mutation urgently need a medical intervention that prevents all types of BRCA-related cancer and leaves people whole and healthy. This is the goal of the HeritX Foundation. As a first step, HeritX assembled experts across a wide range of topics to collectively design a research agenda with actionable short-term milestones toward the prevention of BRCA-related cancer.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Defining the Goal of Preventing Inherited BRCA Cancer

Chairperson: T. Bock, HeritX, Chester, New Jersey

The HeritX Global Research Initiative for Preventing Inherited Cancer
T. Bock, HeritX, Chester, New Jersey
Preventing Inherited BRCA Cancer: A Think Tank for Innovative Strategies, Milestone Objectives

A. Ashworth, University of California Cancer Center, San Francisco
L. Brody, National Human Genome Research Institute, NIH, Bethesda, Maryland

Integrated R&D Planning Leads to Faster Patient Benefit
D. Hager, HeritX, Chester, New Jersey

General Discussion

Eliminating the Needs of Affected Families
J. Morris, HeritX, Inc., Santa Monica, California (Facilitator)
I. Bock, Chester, New Jersey
P. Munster, University of California, San Francisco
M. Unger, Los Angeles, California

Translating the Patient Goal into a Target Therapy Profile for Preventing Inherited BRCA Cancers
D. Hager, HeritX, Chester, New Jersey

SESSION 2: How Can We Accomplish a Prevention of All Types of Inherited BRCA Cancer?

Chairpersons: L. Brody, National Human Genome Research Institute, NIH, Bethesda, Maryland, and T. Bock, HeritX, Chester, New Jersey

BRCA Overview: The Genes, Proteins, and Their Cellular Roles
R. Scully, Beth Israel Deaconess Medical Center, Boston, Massachusetts

Tackling Seemingly Insurmountable Scientific Challenges: “Think Different!”
M. Olson, University of Washington, Seattle

Discussion

Breakout Group A: Banbury Conference Room
Facilitator: L. Brody, National Human Genome Research Institute, Bethesda, Maryland

Breakout Group B: Meier House Library
Facilitator: W. Foulkes, Montreal General Hospital, Quebec, Canada

SESSION 3: Overcoming Current Hurdles. The Biology of Risk: Identifying the First Steps of Cancer Development in Heterozygous BRCA Mutation Carriers for Therapeutic Targeting

Chairpersons: B. Ponder, CRUK Cambridge Institute, United Kingdom, and A. Ashworth, University of California, San Francisco

Research Strategies to Identify Early Steps in Pathogenesis
J. Brugge, Harvard Medical School, Boston, Massachusetts

Discussion

Technology to Pursue These Strategies in Healthy BRCA Carriers
P. Spellman, Oregon Health & Science University, Portland, Oregon

SESSION 4: Pre-empting Future Hurdles: Surrogate Endpoints—Developing Candidate Therapies Faster

Chairpersons: J. Garber, Dana-Farber Cancer Institute, Boston, Massachusetts, and D. Parkinson, New Enterprise Associates, Palo Alto, California

Research Strategies: Identifying Biomarkers or Bio-Signatures That Could Become Surrogate Endpoints
S. Domchek, University of Pennsylvania, Philadelphia

Non-Cancer Manifestations in BRCA Carriers: Do They Exist? Are They Clinically Meaningful? How To Study?
J. Garber, Dana-Farber Cancer Institute, Boston, Massachusetts

SESSION 5: Implementing Banbury Outcomes after Banbury

Chairperson: T. Bock, HeritX, Chester, New Jersey
A. Ashworth, University of California Cancer Center, San Francisco
L. Brody, National Human Genome Research Institute, Bethesda, Maryland

Conclusion of the Workshop
How Can the Genetics and Neurobiology of Borderline Personality Disorder Contribute to Its Diagnosis and Treatment?

November 15–18

FUNDED BY Oliver Grace Fund, Matthew Warren Fund for Mental Health; The Menninger Clinic; Mt. Sinai School of Medicine; NEA-BPD; and The Soref Family

ARRANGED BY J. Oldham, The Menninger Clinic, Houston, Texas
A. New, Mount Sinai School of Medicine, New York

Borderline personality disorder (BPD) is a serious and disabling psychiatric disorder that is the most prevalent personality disorder in psychiatric treatment populations. Much is now known about the neurobiology and pathophysiology of BPD, and evidence-based treatment strategies have been demonstrated. A great deal of work remains to be done, however, to harness new strategies such as genomics and neuroimaging to identify BPD-specific neuropathology. Biomarkers are needed to facilitate early identification and prevention of BPD, and to monitor its course and treatment. This conference was intended to explore areas such as temperament, emotion regulation, developmental attachment and its disruptions, impulse control, and interpersonal functioning and to collectively design a new research agenda for BPD.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Conference Introduction and Structure: J. Oldham, The Menninger Clinic, Houston, Texas
A. New, Mount Sinai School of Medicine, New York
SESSION 1: Phenomenology of Borderline Personality Disorder

Chairperson: J. Oldham, The Menninger Clinic, Houston, Texas

J. Oldham, The Menninger Clinic, Houston, Texas: Dimensional vs. categorical diagnosis of BPD and the alternative model.

A. Skodol, University of Arizona College of Medicine, Phoenix: Back to the future: BPD Research Foundation Redux.

M. Zanarini, McLean Hospital, Belmont, Massachusetts: The long-term course of BPD.

P. Tyrer, Imperial College London, United Kingdom: How the new classifications of personality disorder might help the genetics and neurobiology of that very heterogeneous condition, borderline personality disorder.

C. Sharp, University of Houston, Texas: The social-cognitive basis of BPD: A translational approach.

Discussion and Summary

SESSION 2: Family Perspective

P. Hoffman, National Education Alliance for Borderline Personality Disorders, Mamaroneck, New York

V. Porr, TARA Association for Borderline Personality Disorder, New York

K. Warren, Acts of Mercy Foundation, Rancho Santa Marguerita, California

SESSION 3: Genetics of BPD

Chairperson: D. Goldman, National Institute on Alcohol Abuse & Alcoholism, Rockville, Maryland

D. Goldman, National Institute on Alcohol Abuse & Alcoholism, Rockville, Maryland: Chemogenetics for addictions: Challenges and opportunities.

M. Distel, GGZ in GeestIVU Medical Center, Amsterdam, The Netherlands: Genetics of BPD: What twin studies have learned us and what is left to learn.

M. Perez-Rodriguez, Mount Sinai School of Medicine, New York: Current status of genetic research in borderline personality disorder.

Sh. Purcell, Icahn School of Medicine at Mount Sinai, New York: Progress and prospects in neuropsychiatric genetics: Lessons learned from schizophrenia and other disorders.

Session Discussion and Summary

SESSION 4: Brain Circuits

Chairperson: A. New, Mount Sinai School of Medicine, New York

A. New, Mount Sinai School of Medicine, New York: An illness of impaired emotional interoception.

C. Schmahl, Central Institute of Mental Health, Mannheim, Germany: How neuroimaging can be used to improve psychotherapy for emotion dysregulation.

Session Discussion and Summary

SESSION 5: Animal Models

Chairperson: J. Oldham, The Menninger Clinic, Houston, Texas

M. Bohus, Central Institute of Mental Health, Mannheim, Germany: Toward an animal model for aspects of BPD: The peer group rejection paradigm in mice.
S. Russo, Icahn School of Medicine at Mount Sinai, New York: Can we model axis II pathology in mice to identify circuit abnormalities?

Session Discussion and Summary

SESSION 6: Treatment

Chairperson: R. Kissell, University of California, Los Angeles, Semel Institute, Beverly Hills

R. Kissell, University of California, Los Angeles, Semel Institute, Beverly Hills, California: Treatment for BPD: What we have and what we need.

B. Stanley, Columbia University, New York: Mechanisms of action of treatment for BPD: What we know and what we don’t know.

M. Goodman, Bronx VA Medical Center, Bronx, New York: The neurobiology of DBT treatment response.

Session Discussion and Summary

SESSION 7: Wrap-up and Future Directions

Moderators: A. New, Mount Sinai School of Medicine, New York, and J. Oldham, The Menninger Clinic, Houston, Texas
Tumor Cell Metabolism: Finding New Targets for Therapeutic Intervention

December 7–10

FUNDED BY Oliver Grace Cancer Research Fund and OBX, Inc.

ARRANGED BY L. Cantley, Weill Cornell Medical College, New York
S. McKnight, University of Texas Southwestern Medical Center, Dallas

Recent research has provided many insights into the biochemical basis for alterations in the metabolic state of tumors compared to normal tissues. This growing body of knowledge about tumor metabolism has revealed new targets for pharmaceutical intervention, and several new experimental drugs that target metabolic enzymes have entered clinical trials. Participants reviewed new metabolic targets, discussed biomarkers that are likely to predict which tumors are likely to respond to drugs that hit these targets, examined potential mechanisms of resistance to such therapies, and discussed drug combinations that could prevent resistance.

Welcoming Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Opening Remarks: J.D. Watson, Cold Spring Harbor Laboratory

SESSION 1: Hypoxia

Chairperson: J.M. Bishop, University of California, San Francisco
R. Bruick, University of Texas Southwestern Medical Center, Dallas: Regulation of hypoxia-inducible factor 2: Sensing opportunities.

J. Josey, Peloton Therapeutics, Inc., Dallas, Texas: Modulation of hypoxia-inducible factor-2α.
W. Kaelin, Dana-Farber Cancer Institute, Cambridge, Massachusetts: New targets emerging from studies of VHL and IDH.
SESSION 2: Addressing Cancer Metabolism in the Clinic
Chairperson: J.M. Bishop, University of California, San Francisco
R. DeBerardinis, University of Texas Southwestern Medical Center, Dallas: Metabolic heterogeneity in human lung tumors.
M. Dorsch, Agios Pharmaceuticals, Cambridge, Massachusetts: Development of mutant IDH inhibitors from concept to clinic.
E. Maher, University of Texas Southwestern Medical Center, Dallas: Metabolic reprogramming of bioenergetic substrate utilization: When does it occur?

SESSION 3: Signaling Pathways That Control Metabolism
Chairperson: H. Varmus, Weill Cornell Medical College, New York
M. Brown, University of Texas Southwestern Medical Center, Dallas: Scap: Sterol sensor and SREBP regulator.
B. Manning, Harvard University, Boston, Massachusetts: Oncogene control of lipid and nucleotide metabolism.
J. Blenis, Weill Cornell Medical College, New York: New mechanisms for mTORC1-dependent regulation of cell metabolism.
L. Cantley, Weill Cornell Medical College, New York: Managing ROS in cancer cells.
A. Kimmelman, Dana-Farber Cancer Institute, Boston, Massachusetts: Identifying metabolic vulnerabilities in pancreatic cancer.
T. Miller, IC MedTech Corp, El Cajon, California: Vitamin C selectively targets cancer cells: Can combining other redox-active molecules and chemotherapy translate into safer, more effective, and more affordable clinical protocols?
K.-L. Guan, University of California, San Diego: The Hippo pathway in cellular nutrient response.
R. Shaw, Salk Institute for Biological Studies, La Jolla, California: The LKB1–AMPK pathway: Metabolic rewiring and therapeutic targeting.

Y.-S. Lee, Johns Hopkins University, Baltimore, Maryland: Structure and functional analysis of dimeric PKM2.

H. Christofk, University of California, Los Angeles: Use of viruses to study cancer metabolism.

R. Evans, Salk Institute for Biological Studies, La Jolla, California: Stromal regulation of pancreatic cancer epigenome and metabolome.

**General Discussion Highlighting Key Points**

**SESSION 4: Cellular Control by Metabolic Intermediates**

Chairperson: A. Levine, Institute for Advanced Study, Princeton, New Jersey

K. Vousden, Beatson Institute, Glasgow, United Kingdom: One carbon metabolism in cancer cells.

B. Tu, University of Texas Southwestern Medical Center, Dallas: Intracellular pathways responsive to SAM.

J. Rutter, University of Utah School of Medicine, Salt Lake City: The impact of pyruvate metabolism on stemness in normal and cancer settings.

J. Locasale, Duke University, Durham, North Carolina; One carbon metabolism and epigenetics.

S. Gross, Weill Cornell Medical College, New York: Untargeted stable isotope tracing to define the contribution of serine to folate-mediated 1-carbon trafficking.

**General Discussion Highlighting Key Points**

**SESSION 5: Alternative Nutrients for Cancer Cells**

Chairperson: A. Levine, Institute for Advanced Study, Princeton, New Jersey

E. White, The Cancer Institute of New Jersey, New Brunswick: Autophagy and cancer metabolism.


L. Trotman, Cold Spring Harbor Laboratory: Mitochondria and dietary control of prostate cancer.

G. Smolen, Agios Pharmaceuticals, Cambridge, Massachusetts: Differential aspartate usage identifies a subset of cancer cells particularly dependent on a novel metabolic target.

**SESSION 6: Review of Key Issues**

Chairpersons: L. Cantley, Weill Cornell Medical College, New York, and S.L. McKnight, University of Texas Southwestern Medical Center, Dallas

Closing Remarks:

J.D. Watson, Cold Spring Harbor Laboratory

H. Christofk, J. Watson
In the summer, a life-size replica of Ötzi (“the Iceman”) arrived at the DNA Learning Center (DNALC). He became the newest member of a cast of prehistoric characters that we use to interest students in human evolution, including an articulated Neanderthal skeleton and burial cast; a bust recreation of *Homo erectus*; and skulls of *Australopithecus afarensis* (“Lucy”), *Homo habilis* (“Handy Man”), *Homo floresiensis* (the “Hobbit”), and other ancient hominids. These exhibits are designed to complement experiments, pioneered by the DNALC, in which students use their own DNA as an entrée to the study of human origins.

Ötzi took the center stage in the DNALC’s main exhibit hall, backed by a wall-sized graphic of the mountainous site in the Ötztal Alps where he was discovered in 1991. The Ötzi installation—which includes recreations of clothing, weapons, and other artifacts discovered along with the mummy—is funded by a grant from the Long Island Real Estate Group.

Like our hugely successful interpretive program, “Mystery of Anastasia,” Ötzi provides a fascinating exploration of forensic science. On one level, Ötzi has the classic elements of a murder
mystery: An arrow point entered through his shoulder blade, severing his subclavian artery and causing him to bleed to death in several minutes. What was he doing at 10,500 feet on the mountain? Who followed him to that altitude and shot him from behind? Whose blood was found on his clothes? What were they fighting about?

On another level, Ötzi tells stories about human evolution. He is dated to 5300 years before present, a time when agriculture was sweeping through Europe. The basic DNA types he inherited on his maternal and paternal sides originated in western Asia and spread through Europe with farmers who carried farming out of the Fertile Crescent. Analysis of his stomach contents showed that Ötzi’s last meal included unleavened bread made with cultivated einkorn wheat, which had arrived in northern Italy from its origin in southeastern Turkey about 2000 years before Ötzi’s time. However, Ötzi did not have the gene mutation that helps adults digest milk sugar, which moved south from its origin in northern Europe.

After a year of negotiations, the DNALC received exclusive rights to display three-dimensional (3D) replicas of Ötzi at the Dolan DNALC and in its future center in New York City. These will be the only authorized replicas to be displayed outside of Ötzi’s home in the South Tyrol Museum in Bolzano, Italy. The replicas were produced by Gary Staab (www.staabstudios.com), the leading artist specializing in natural history and prehistoric life models for museums. Raw data from a full-body computerized tomography (CT) scan were used to develop a 3D representation of the surface of Ötzi’s body. Then, in February, these data were used to make a 3D print on a mammoth stereolithography machine at Materialise in Leuven, Belgium. The print was then shipped to the Staab Studios in Kearney, Missouri, for finishing. Although the 3D printing was highly accurate, it did not have enough resolution to define skin texture and features; these were painstakingly added by hand with reference to a photographic atlas. Using the “lost wax” process, a rubber mold was created from which durable resin casts were made. Gary then hand painted the entire cast to add skin details, including a number of tattoos.

**CyVerse (iPlant Collaborative)**

CyVerse, a contraction of “cyber universe,” is the new name for the National Science Foundation (NSF) project that began as the iPlant Collaborative in 2008. CyVerse provides researchers and educators the high-performance computing, databases, software, and cyberinfrastructure to store, share, and analyze the ever-growing "big data" of biology. The DNALC-led Education, Outreach, and Training (EOT) component of CyVerse focuses on bringing data science into classrooms and
Data science is an interdisciplinary field where raw data from multiple sources is integrated and transformed into knowledge. It is used in many fields and increasingly affects our daily lives. For instance, data analysis from multiple sources informs shopping recommendations and provides real-time traffic alerts. Data science also improves health outcomes and allows researchers to study diverse biological problems, including the relationships between genotype and phenotype, gene networks, or entire ecosystems. Building the DNALC’s investment in computation, we hired Joslynn Lee as our first data science educator. With a Ph.D. in computational chemistry, Dr. Lee guides biologists and biology educators as they enter the powerful, but often daunting, world of data science.

Bringing Data Science to Biology: We continued to work with Software Carpentry (www.software-carpentry.org), a nonprofit organization of volunteer instructors whose mission is to teach basic computing skills to scientists and students. Jason Williams is a certified Software Carpentry instructor and a member of their 2015 Steering Committee. We also worked with Data Carpentry (www.datacarpentry.org), which started as collaboration between NSF-funded BioCenters, including CyVerse. Data Carpentry teaches skills and best practices of data science in biology. Lessons from both projects are integrated into our CyVerse training.

Use of DNA Subway, our classroom-friendly gateway for gene and genome analysis, increased substantially. Visits increased 26% to 55,755 sessions, new user registrations climbed 13% to 3983, and new projects increased 13% to 28,938. This year, the Green Line (RNA-Seq) was updated to use CyVerse's new API Agave, the interface to high-performance computing resources for data science. Although initial testing was promising, heavy usage and slowdowns during summer workshops revealed the need for further improvement. At the close of 2015, we started exploring seamless access to supercomputers at the Texas Advanced Computing Center (TACC) and Amazon Web Services for the Green Line, with the aim of supporting large numbers of simultaneous projects without disruption. Planning also began on a new classroom-friendly workflow for metagenomics and microbial genomes. This workflow will allow students to barcode complex mixtures, rather than single species, using next-generation sequencing (NGS) to generate sequences of ribosomal 16S RNA.

Workshops, Training, and Events: In 2015, we held six 2-day Tools and Services Workshops, attended by 185 researchers, three 2-day Genomics in Education Workshops for 54 educators, and a Train-the-Trainers workshop for 25 participants from the USDA Agricultural Research Service. In March, the DNALC hosted a Data Carpentry hackathon on “Genomics Lessons and Assessment” at CSHL. Twenty-six participants, including beginners and experts from the United States and abroad, worked for 3 days on lessons designed to introduce scientists to genomics. These lessons teach bioinformatics skills while emphasizing how to avoid common pitfalls that thwart biologists new to computational projects. They were introduced at Data Carpentry workshops held at the American Society of Plant Biology’s annual meeting in Minneapolis, University of California, Davis, and Stony Brook University. We had our biggest showing ever at the 23rd International Plant and Animal Genome meeting in San Diego with an outreach booth and nine sessions and workshops attended by more than 500 visitors. As a kickoff to working with iPlant UK (a collaboration between the Universities of Warwick, Liverpool, Nottingham, and the Genome Analysis Centre), we hosted a workshop in the United Kingdom. We also presented at the Bioinformatics Open Source Conference, as well as at the concurrent Conference on Intelligent Systems for Molecular Biology (ISMB) and European Conference on Computational Biology. These international collaborations point to the global success of CyVerse and the scope of its outreach programs,
including DNA Subway. With our help, 62% of workshop attendees hear about CyVerse from their colleagues. CyVerse has now surpassed the 30,000-user mark.

We have continued to complement in-person workshops with virtual training and outreach. Through Livestream, our online broadcast of Tools and Services Workshops had 1247 views, and our live monthly Get Started with CyVerse webinars attracted 146 participants. Our recorded workshops, webinars, and tutorials on YouTube increased 53% to 16,252 views (more than 1202 hours of content viewed).

Bringing RNA Sequence Data Analysis into the Undergraduate Classroom

Not all genes are transcribed into RNA all the time: Only a subset is expressed in any cell at any given time. For instance, changes in gene expression can change a normal to a disease state. Therefore, determining which RNAs change expression level—differential gene expression—is critical information for researchers trying to unravel many biological questions. The latest technology to determine the transcriptome—the expression level and identity of RNAs in a sample—is RNA sequencing (RNA-Seq).

Unfortunately, most faculty members at primarily undergraduate institutions (PUIs) hope to teach about and use RNA-Seq, but they lack the training and resources to do so. In 2014, we launched a 3-year, $537,000 National Science Foundation (NSF)-funded project, “Infrastructure and Training to Bring NGS Analysis into Undergraduate Education,” to support training and implementation of RNA-Seq by PUI faculty. The project supplies faculty otherwise cost-prohibitive sequencing and leverages free access to supercomputing power and data storage through the “Green Line” of DNA Subway and CyVerse resources.

We hosted our first workshop in 2014, when 10 Working Group members learned to analyze RNA-Seq data and helped develop an RNA-Seq curriculum. Building on these efforts in 2015, we trained 33 faculty members, from 32 PUIs in 18 different states, at regional workshops held at Bowie State University in Maryland and California State University at Long Beach. Each 5-day workshop included seminars and computer demonstrations that introduced RNA-Seq experimental goals and data analysis theory, and participants planned and conducted bioinformatics analyses with assistance from DNALC staff and expert guest lecturers.

A major benefit for program participants was the acquisition of novel RNA-Seq data supporting their research interests. Twenty-five participants collectively received a total of 2.9 billion 76-base-pair paired-end DNA sequencing reads covering 100 transcriptomes for 12 different eukaryotic species. Their diverse projects included characterization of gene expression changes upon exposure of Saccharomyces cerevisiae to copper, during differentiation of human leukemia cells, and in the Drosophila embryonic central nervous system in response to trauma. To accommodate data analysis, 10 new species’ reference genomes were added to the Green Line, which includes genomes from model genetic organisms, such as mouse and Arabidopsis, and many nonmodel organisms, including lizard, honeybee, and chicken. During the workshops, 1800 data analysis jobs were processed on up to 12 processors each, consuming 38,400 CPU-hours.

To date, more than 400 students were taught RNA-Seq by program participants in courses including genetics, developmental biology, cell and molecular biology, and horticulture. Project goals and data were also disseminated through oral and poster presentations by participating faculty and their students at 18 national or regional scientific conferences.
We also added content to our popular (7639 visitors in 2015) RNA-Seq for the Next Generation website (www.rnaseqforthenextgeneration.org). This website features a dedicated page for each faculty member involved in the project, including a description of their research area, links to their RNA-Seq data, and educational resources they have developed.

**Genomic Approaches in Biosciences: Final Year**

The year saw the completion of the last two workshops for our NSF Advanced Technological Education (ATE) faculty training program—one at the University of California, San Francisco, led by Bruce Nash, and one at Southwestern College in Chula Vista, California, led by Thomas Tubon, from Madison Area Technical College. During the project, 302 faculty members attended week-long workshops on key technologies of modern biology conducted by DNALC instructors, whereas 126 attended workshops led by community college faculty who were trained by DNALC staff.

Preliminary results of longitudinal evaluations designed to assess the long-term impact of the program were presented at ATE’s annual Principal Investigator meeting in Washington, D.C. In addition to pre- and postworkshop surveys for 2015 attendees, 9–12-month and long-term 15+ month follow-up surveys were administered to previous workshop participants, revealing that the vast majority of participants (92.7%) have used the materials as background information or student resources, to train fellow faculty, and for teaching. Respondents reported student exposures to labs (21,861), bioinformatics exercises (11,287), and biotechnology career modules (8185) in schools with an average of 40% students from ethnic groups underrepresented in sciences.

We also aimed to measure the relative effectiveness of DNALC staff and instructors we trained. When compared, responses to the long-term follow-up surveys show no significant differences with respect to participant confidence in teaching the lab and bioinformatics methods learned in the workshops whether taught by DNALC staff or local faculty. This confirms the usefulness of our “train-the-trainer” model, supporting continued training of educators who can broaden the impact of our programs by training their peers.

**New Developments around the World**

**DNALC Asia**

On February 13, we successfully concluded negotiations to develop a DNA Learning Center Asia in Suzhou Industrial Park (SIP), outside of Shanghai, China. SIP Administrative Committee (SIPAC) Chairman Zhiping Barry Yang signed the agreement, with John Maroney representing CSHL. The objective is to offer the same hands-on lab experiences in Suzhou as offered at the DNALC in Cold Spring Harbor. This feeds on Chinese brand consciousness and the interest in preparing students for science study at American universities.

The agreement is a product of the DNALC licensing program that began in 2002. The idea of capitalizing on DNALC intellectual property was first championed by CSHL Trustee Arthur Spiro, who brokered the first licensed center, DNALC West at North Shore-LIJ Healthcare System (2002), and another at Clemson University (2006). Since then, licensed centers were developed with the Singapore Ministry of Education (2003), University of Vienna (2006), Notre Dame University (2013), Beijing 166 High School (2014), and Mexico City Health Park (2014). During this period, the licensing program has generated more than $1.2 million in license fees and $1.9 million in operating income.
The 10-year contract will provide significant core support to the DNALC—a $1 million signing incentive plus $225,000 in annual license fees. In addition, SIP will provide $2.5 million for facility renovation and equipment and $2.5 million operating support to support DNALC Asia in its first 5 years. Our business plan anticipates that the center will be generating a surplus of revenue over expenses in year 6. Under terms of the agreement, DNALC Asia is registered as a domestic nongovernmental organization (NGO) sponsored by CSH Asia Conferences.

The Chinese government issued the “Certificate of NGO Registration” on July 14. It is an “internal” NGO, sponsored by an organization operating in China, and it has a Chinese legal representative, as opposed to an “external” NGO, such as UNICEF, Doctors Without Borders, or the Red Cross. The NGO is governed by a nine-member council composed of four SIPAC and four CSHL representatives, as well as the CEO of Cold Spring Harbor Asia Conferences, Maoyen Chi, who also serves as the Council Secretary General, the NGO Chairman, and the first-term Director of DNALC Asia. The first council meeting was held in Suzhou on October 21. During the first year, the NGO will share core management and accounting services with CSH Asia Conferences.

DNALC Asia occupies modern “pod-like” buildings in SIP’s Biobay in Dushu Lake Science & Education Innovation District, with a land area of 270,000 square feet. In the first phase, one 20,000-square-foot two-story pod is being built with eight teaching labs to accommodate 224 students—twice the capacity of the Dolan DNALC. An exhibition hall and cafeteria are also included in the total renovation area of more than 30,000 square feet—to be completed in spring 2016. The facility is being renovated to our specifications, including signature DNALC lab benches that encourage educator and student interaction. To provide an “American” lab experience, each large Chinese class will be split into two groups. The business plan calls for bringing the second pod online in year 4.

In July, a small SIPAC delegation visited the Dolan DNALC. The group included Fang Xia, Vice Chairman of SIPAC; Shaojie Xu, Deputy Director of the Education Bureau; and Jianguo Yin, Deputy Chief of the International Cooperation Division of Science & Technology Development Bureau. In addition to meetings on DNALC operations and the proposed 3D Cellarium exhibition as rendered by Centerbrook Architects and Planners, the group observed summer camps for local and Beijing 166 students and met Dr. Watson. We also hosted a group of 16 tenth grade students (plus two adults) from SIP schools as part of a 2-week U.S. summer study program. This collaborative initiative was organized by Suzhou Dushu Lake Science & Education Development Company and authorized by the SIPAC Education Bureau. The group performed a DNA
fingerprinting lab, joined a talk with Dr. Watson at the DNALC, and then toured the CSHL campus.

Recruitment of two international and three domestic Chinese instructors for DNALC Asia started in late April. We advertised the International Faculty Teaching Fellowships primarily by email, targeting young postdocs. Newly hired faculty will be trained at the DNALC in early 2016, prior to the anticipated opening of DNALC Asia in spring. In addition, Lilly Wang, DNALC Asia Promotion Manager, and Dorothy Zhang, DNALC Asia Operations Manager, spent time at the DNALC in July and December, respectively, to be trained in DNALC programming, school recruitment, and administration.

Beijing No. 166 School

The importance of quality science education in China was highlighted this year by recommendations from the Beijing Education department to increase middle school science content. The licensed DNALC at the Beijing No. 166 School will enrich experiences for students and teachers from the entire Dongcheng District as they implement mandated lab experiments for seventh and eighth grade students and a required biology course for ninth graders. Plans to use the lab space as a hub for teacher training, footlocker kit rental, student field trips, and a Beijing DNA Barcode Project were developed this year, while contracted DNALC exchange programs for the students of the Beijing No. 166 School continued.

Two cohorts of Beijing students came to the DNALC for workshops in the winter and summer. In February, 38 high school students attended two week-long courses. In DNA Science they learned basic techniques of recombinant DNA in bacteria. During week 2, they progressed to biochemical and computer methods to analyze DNA barcodes of plants and animals. Two students from the group were selected to conduct a 2-week independent research internship with DNALC staff, during which they designed and conducted projects to study their own microbiomes using DNA barcoding and traditional microbiology techniques. The experience culminated with oral presentations of research findings and laboratory experiences. In July, 38 middle school students

In February, Beijing No. 166 School students met with Dr. Watson (left) and performed DNA barcoding laboratories in the Hershey teaching lab on the CSHL campus (right).
visited for 3 weeks and completed labs from popular middle school camps *Fun with DNA*, *World of Enzymes*, *Green Genes*, and *Forensic Science*. The students were immersed in hands-on activities and laboratory experiments designed to increase genetic literacy, encourage critical and creative thinking, and spark interest in the field of biotechnology. At the same time, four middle school science teachers from Beijing 166 began training in basic lab techniques, including DNA barcoding.

During the spring and fall, DNALC staff visited Beijing to conduct in-school workshops. In April, 175 high school students and 30 high school science teachers from the Beijing area extracted and amplified their DNA to detect the presence of an *Alu* insertion as part of a *Human DNA Fingerprinting* workshop. In October, 32 middle school students completed labs on inheritance and variability, DNA structure and isolation, DNA transformation and protein isolation, and DNA restriction analysis in the *Introduction to Genetics and Biotechnology* Workshop. In addition, a workshop for 24 teachers introduced 24 Beijing high school science teachers to DNA barcoding research, and 24 ninth grade students completed a short *DNA Barcoding Research* course. DNALC teaching activities in Beijing were reported by Chinese newspapers and TV, promoting the “learn by doing” style of DNALC programs. Our collaboration with the Beijing No. 166 School provides a template as we bring Beijing to the forefront of biology education in China.

**Regeneron**

Regeneron Pharmaceuticals, Inc., is planning to open a licensed DNALC in a new building at its Tarrytown biotechnology complex. Founding Scientist and Chief Scientific Officer of Regeneron Pharmaceuticals, Inc., and President of Regeneron Laboratories, Dr. George Yancopoulos, joined the CSHL Board of Trustees in November. With his research team, Dr. Yancopoulos developed Eylea, a treatment for wet, age-related macular degeneration. Eylea has since become a “blockbuster” drug, generating more than $1 billion in sales for the company. With a commitment to investment in the community, Regeneron plans to use the proposed on-site DNALC to support its own outreach initiatives and a robust school-based field trip program operated by the DNALC. If all goes according to plan, contracts will be signed in 2016, with an opening in 2017.

**Notre Dame**

The DNALC at Notre Dame—made possible by CSHL friend and Notre Dame Alumnus, John Passarelli—is a 36-seat lab in the Jordan Hall of Science. More than 1300 students in grades 5–12 participated in hands-on labs either at the Center or in their classrooms, some from as far as Alaska! Summer camps for 130 students in grades 6–10 included residential and day programs with an average satisfaction rating of 9.5/10 for campers and parents. Capitalizing on the college campus location, more than 50 undergraduate and graduate volunteers were invited to assist at DNALC functions, including a DNA day essay contest with prizes at the elementary, middle, and high school level, and as judges at local-, regional-, and state-level science competitions. Led by Director Amy Stark, the staff doubled this year with the addition of Linda Rock as administrator and second teacher.

**DNA Center NYC**

This year brought us closer to achieving the ongoing goal of a dedicated center in New York City (NYC). We have been working toward a Memorandum of Understanding (MOU) with the City University of New York (CUNY) for rights to develop a DNALC location at the NYC College of Technology (City Tech) in Brooklyn. The 17,500-square-foot space is located on the second floor of the Pearl Street Building, on the corner of Adams and Tillary Streets. The DNALC will have a separate dedicated entrance and lobby on Tillary Street, with elevator and stairway access. We expect the site to become available for renovation in 2017, with operations commencing in 2018.
Under terms of the MOU, we expect to obtain the space lease-free for 30 years. The DNALC would operate two teaching labs for the exclusive use of CUNY students. These labs would support course-based undergraduate research experiences (CUREs) and honors classes on the DNALC’s published curricula DNA Science and Genome Science, which would serve approximately 500 CUNY students per year.

CSHL would benefit from shared use of CUNY facilities (such as large auditoriums and conference spaces), maintenance, and security. The City Tech property fulfills CSHL’s key requirements of visibility and ready access to the student populations of NYC. With signage on the facade of the building, the DNALC brand will be immediately visible to all traffic coming off the Brooklyn Bridge. Downtown Brooklyn is one of the most accessible parts of NYC, and there are eight subway lines within several blocks of City Tech. There is ample room for bus drop-off on both Tillary and Adams Streets.

This location would provide a local audience for summer camps, a crucial component of the DNALC NYC business model; it is also easily accessed via public transportation by students throughout NYC. Brooklyn is the fastest-growing of the five boroughs and has the largest population of school-age children (~400,000) and students enrolled in public school (~300,000). Brooklyn has the greatest number of charter schools and highest charter school enrollment (~18,000). To support programs in NYC, including at our new location, we finalized a business contract with the NYC Department of Education (NYCDOE). This Multiple Task Award Contract (MTAC) allows us to provide field trips, in-school instruction, summer camps, and professional development as a vetted contractor, removing the lengthy bidding process usually required for vendors in NYC. We also aim to provide programs for students attending independent schools near the proposed location, which include St. Ann’s School, Packer Collegiate Institute, Brooklyn Friends School, Berkeley Carroll School, and Poly Prep Country Day School.

DNA Barcoding

The DNALC’s three DNA barcoding programs continue to enable authentic research by students while contributing to our knowledge of biodiversity: The National Institutes of Health (NIH)-funded Barcode Long Island (BLI) enables students to study the biodiversity of Long Island with teachers as mentors; the Urban Barcode Project (UBP) similarly supports student teams in NYC; and the Urban Barcode Research Program (UBRP), supported by the Pinkerton Foundation, trains students in conservation biology and DNA barcoding and then pairs them with scientists who mentor their research.

A measure of the success of these programs is the effect on the attitudes of participants. For example, interviews conducted with BLI students highlighted the hands-on nature of the activities and the discovery qualities inherent in doing “real science.” One BLI student said, “I think my favorite part was going out and actually collecting the lichens and getting some hands-on experience rather than just learning about it in the classroom.” Another student told interviewers, “It was nice to make our own science project rather than do a lab in a classroom where it is set, and you know the results at the end. We’re basically like scientists and making our own procedure.” The interviews also illuminated some of the challenges of BLI and broader lessons: “This is going to help me in the future because I feel that it teaches me to continue on even though sometimes you might fail at first, or a second time, or a third time, you keep on continuing because eventually you get some good results.”
To better understand the trends among our DNA barcoding students, data for all three programs (BLI, UBP, and UBRP) were combined and analyzed. Among students with other research or science competition experiences ($n = 83$), the majority (57.8%) felt that the DNA barcoding programs provided much more “real world” science and chances for hands-on experiences than their previous experiences had. These students also indicated that they had many more opportunities to find out something new (42.2%), and to develop critical thinking and understanding of the scientific process (34.9%), while being more fun than other research experiences (34.9%). More than half of students from all three programs expressed an increased interest in a career in science, with UBRP students having the highest level of increased interest (87% for UBRP vs. 68% for UBP and 50% for BLI). As a whole, these results suggest that DNA barcoding research programs are more effective than other popular research experiences, especially when students are mentored by experts.

DNALC barcoding mentors, whether teachers or researchers, enjoyed working with high school students. As one UBRP mentor noted, “The most enjoyable aspect of the Urban Barcode Research Program was working with and learning from young bright, enthusiastic students working within a lab environment and applying real world solutions to real questions. Even negative results are results and thus a learning experience.” Many mentors indicated their experience had a positive impact on their professional development; assisting students with proposals (73%) and completing bioinformatics exercises (81%) were perceived to be of great professional benefit.

To support student efforts and archive their findings, we continued to improve our barcoding websites, which include background information, proposal submission guidelines, and portals for projects. We improved the content of DNA Barcoding 101 (www.dnabarcoding101.org), including detailed instructions, background, and support materials on DNA barcoding. We enhanced the proposal submission, review, revision, and acceptance system, features necessary to make large-scale, distributed student DNA barcoding feasible. The sites now handle teacher requests for reagents and equipment, announcements and registration for training and “Open Labs,” and directions for the Survey of Undergraduate Research Experiences (SURE-III) student survey, encouraging participation in our assessment of the programs. The barcoding sample database was also enhanced to allow students to upload multiple pictures of samples, sampling area, and polymerase chain reaction (PCR) results. A system for reviewing PCR results and sequencing requests was also automated. At year’s end, we began discussions to merge our DNA barcoding websites with the aim of producing a scalable platform to support and host student DNA barcoding programs beyond the region.

The Blue Line of DNA Subway (http://dnasubway.iplantcollaborative.org), which integrates tools for DNA barcoding analysis in a simple, accessible workflow, continued to be optimized to support DNA barcoding. The Blue Line workflow was enhanced by adding outgroups to phylogenetic analyses, and dedicated support for fungal (ITS) and microbial (16S ribosomal RNA hypervariable region) barcodes is currently being developed and will be available in early 2016.

Barcode Long Island: Exploring Biodiversity in a Unique Urban Landscape (BLI) is funded by a $1.2 million NIH Science Education Partnership Award. The project provides teacher training, reagents, equipment, and technical support for students to carry out DNA barcoding research at their own schools or during Open Labs held at DNALC (7), BNL (4), and SBU (3). In the spring, 78 students in 29 teams supported by 22 teachers from 20 schools in Nassau, Suffolk, and Queens Counties completed projects and presented results at the inaugural BLI Symposium in June. An average of 20 DNA barcoding samples was processed by each team, yielding 467 barcodes for projects on plant (50%), lichen (13%), invertebrate (33%), and fungal (4%) biodiversity.

A planning meeting for all collaborators and stakeholders was held at CSHL on June 5. Collaborators from Stony Brook University (SBU), Brookhaven National Lab (BNL), the American Museum of Natural History (AMNH), Dowling College, Molloy College, Brandeis University, Brooklyn College, Genspace Community Biolab, and Suffolk County Water Authority were joined by teacher
fellows and DNALC project staff. In addition to supporting teams directly at SBU and BNL, scientists at SBU, the AMNH, Malloy College, and Dowling College are helping to identify species using traditional taxonomic methods. The AMNH has agreed to archive invertebrate specimens with novel barcodes, and Brooklyn Botanic Garden scientists are considering archiving plant specimens.

In the summer, 93 teachers were trained during three 5-day workshops held at the DNALC, SBU, and BNL and a 1-day workshop at the DNALC in the fall. Of the 144 teachers trained in the first 2 years of the project, 41 are now mentoring 105 teams composed of 329 students representing 34 public Long Island schools. At the close of 2015, this second cohort of students is studying diverse ecosystems and taxa, including terrestrial and aquatic animals, plants, fungi, and lichens across Long Island.

Meanwhile, in NYC, the Urban Barcode Project remains strong. On May 27, 50 teams presented project findings at a poster session at the AMNH. Research included mapping wildlife in NYC, detecting food fraud, determining biodiversity of NYC green spaces, identifying traded plant and animal species, and exploring the effects of biodiversity on human health. A jury of 29 experts in biodiversity, conservation biology, DNA barcoding, and education selected seven finalist teams who gave oral presentations at the AMNH on June 8. Susan Perkins, Curator and Professor at the Sackler Institute for Comparative Genomics and Division of Invertebrate Zoology at the AMNH, gave the keynote talk “Meet Malaria's Mates: The Use of DNA Data to Discover and Describe New Species of Parasites.” UBP grand-prize winners Armani Khan and Jessica Kuppan, mentored by Karen Wong, are from the High School for Construction Trades, Engineering, and Architecture in Queens. They used DNA barcodes to identify macroinvertebrate bioindicators in Queens parks and plan further research on how to conserve and rehabilitate natural areas. In the fall, 70 student proposals were submitted and 55 teams—175 students—have been accepted into the program for the 2015–2016 academic year.

The Urban Barcode Research Program symposium was held on June 15 at the AMNH, and 20 teams composed of 39 students presented posters and gave oral presentations. These students were selected from 130 high-achieving NYC high schools that completed UBRP preparatory courses. The students worked with 19 scientist mentors from 14 NYC institutions, including the AMNH, NYU, CUNY, Fordham University, and Albert Einstein Medical Center. UBRP projects spanned studies of food fraud, biodiversity, conservation genetics, phylogenetics (evolution), forensics, and metabarcoding. Example projects included assessing the diversity of aquatic plants and invertebrates in freshwater ecosystems of Staten Island and investigating Salmonella bacteria in captive reptiles and amphibians found in NYC. The students’ projects were remarkably sophisticated, and
mentors indicated that data from at least 11 of these studies will either contribute to or result in publications. This year’s student cohorts represented 30 public and 10 private high schools from the New York metropolitan area. Nearly one-third of participants were African–American or Latino, groups underrepresented in science.

Sixty-seven students attended Open Lab sessions at the Harlem DNA Lab or Genspace in Brooklyn, and 95 students used borrowed equipment footlockers to complete their research. Teams collected and processed more than 1200 samples for DNA sequencing—an average of 17 samples per team—with more than 1600 single sequences provided by GENEWIZ, Inc., and one million reads produced by next-generation sequencing. Importantly, UBP and UBRP students produced 35 novel DNA barcodes. These are being published with the students as authors in the international database, GenBank, to make the data available for use by other researchers.

Outside of these large programs, we continued our partnership with the NYC DOE to offer a 2-week DNA barcoding course at the Environmental Study Center (ESC) in Brooklyn, where teams from four public high schools assessed the biodiversity of Brooklyn’s Marine Park. The students collected 92 samples, with six novel sequences published to GenBank. Most of the barcoding sequences were from plants, ants, and aquatic invertebrates. One team continued research in the UBP during the academic year to further study ants and macroinvertebrate biodiversity in Queens’ salt marshes. Five of nine teachers trained through this collaboration at ESC have continued as mentors, either as part of the UBP or through sponsorship of after-school programs on DNA barcoding at their schools.

This summer, 37 middle school students attended Backyard Barcoding, a 1-week introduction to DNA barcoding and how it can be used to identify species. An additional 13 students attended the 2-week DNA Barcoding Research camp, where students design their own research projects to study biodiversity.

Student Programs

In 2015, 20,570 students attended field trips at our three facilities: Dolan DNALC, DNALC West, and Harlem DNA Lab. An additional 8908 students completed labs in-school led by DNALC staff, and 1348 students attended week-long summer camps.

Grants from Bank of America and National Grid Foundation supported programs for more than 2500 underserved students from Long Island public school districts including Brentwood, Uniondale, William Floyd, Central Islip, and Valley Stream. An additional 17 students from the ZION STEM Academy of Elmont and 24 students from New World Prep Charter School in Staten Island received scholarships to attend the Fun with DNA camp in NYC. Fifteen students from I.S. 59 in Queens also participated in an ongoing collaboration with Northwell Health (formerly North Shore–LIJ Health System) at DNALC West, which was showcased during NYC DOE’s STEMinastic event at the New York Hall of Science.

Of the 2722 students who visited the Harlem DNA Lab, 73% received scholarships partially funded by the William Townsend Porter Foundation, including two schools co-located within the John S. Roberts Educational Complex: the Coalition School for Social Justice and Harlem Village Academy Leadership School. The NYC DOE’s Office of School Programs and Partnerships subsidized camps at the Harlem DNA Lab and Environmental Study Center serving 72 students.

The NYC Partner Membership Program grew in 2015 with the addition of the Lycee Francais and Riverdale Country Schools. The eighth grade research students at Lycee Français and Riverdale Country Schools. The eighth grade research students at Lycee Francais conducted independent research using DNA barcoding to investigate food fraud, followed by a small poster session for classmates and parents. Riverdale Country School plans to offer lab sequences for seventh, ninth, and eleventh grade students in spring 2016. The Chapin School and Marymount School of New York continued to offer the advanced elective Molecular Genetics, which is
co-taught by DNALC instructors. In addition, lab sequences based on scientific inquiry have been implemented in the sixth, seventh, and ninth grade science classes. St. David’s School completed its second year of membership with a fifth grade genetics program, eighth grade independent DNA barcoding projects, and a forensics summer camp in June. Science Research students at the Convent of the Sacred Heart in Greenwich continued DNA barcoding projects, with some teams planning to perform microbiome analysis in spring 2016. Additionally, the Convent of the Sacred Heart continued to offer lab sequences for middle school and high school Life Science, Biology, Advanced Placement Biology, and Environmental Science courses.

The college-level course Molecular and Genomic Biology, co-instructed by DNALC staff and Cold Spring Harbor High School faculty Jaak Raudsepp, continued as part of a long-standing collaboration with the CSH Central School District. Enrolled students spent two periods a day immersed in hands-on experimentation and independent projects, primarily at the DNALC. The 2015 cohorts—our tenth and eleventh classes in this program—have completed units in bacterial genetics, DNA barcoding, bioinformatics, RNAi (RNA interference), eugenics, human and plant genomics, and microbiomes.

St. Dominic High School’s elective course, Molecular and Genomic Biology Research, continued in the spring with independent projects for the third cohort of high school juniors. Some projects used DNA barcoding to study local biodiversity, whereas others chose to work with Caenorhabditis elegans. The fourth cohort started the 5-month instructional portion of the course in September with lab-based introductions to bacterial genetics, DNA barcoding, RNAi, and human genomics.

The Nassau BOCES STEM Institute (formerly Doshi STEM School) finished year 2 by presenting DNA barcoding posters at the BLI symposium. The school welcomed its third cohort of students in September. As part of an initiative to encourage independent research, a small set of tenth graders began experiments using RNAi to investigate gene function in C. elegans.

The footlocker kit program supporting teachers as they lead student research continued to grow in 2015. The program provides equipment and reagents to implement activities including genetics, biotechnology, and DNA barcoding. Footlocker kits were used by 1741 students, 166 of whom were conducting independent research through UBP, UBRP, or BLI; more than 50% were underrepresented minorities.

Monthly Saturday DNA! sessions drew 229 participants, with parents and grandparents joining children for classes on natural selection and the senses, cloning, bacteria, and antibiotics, Gram staining, gel electrophoresis, genetic engineering, crime scene investigation, plant biology, and Ötzi the Iceman. An additional 5421 visitors viewed the DNALC museum exhibition and/or viewed films in the Multitorium, including Cablevision’s multimedia presentation Long Island Discovery.

Graduate Training

Our collaboration with the Watson School of Biological Sciences (WSBS) provides graduate students with the opportunity to learn skills necessary for communicating with nonbiologists. Watson students work with seasoned DNALC instructors to develop presentation methods that can convey unfamiliar ideas to people of any age or background. When the graduate students have completed all three phases of the training, they are prepared to quickly assess audience knowledge and structure and deliver quality lessons.

During the first training phase, Watson students observe a DNALC instructor teaching a laboratory class. Following observation, the graduate students organize a lesson plan that incorporates their knowledge into the class material. In the second phase of training, the graduate students co-teach the class they observed. DNALC instructors provide the Watson students with feedback,
discussing strengths and appropriate preparation for the final phase: teaching the lesson independently under DNALC instructor observation. After completing middle school and high school rotations, Watson students select three additional lessons to demonstrate their new teaching skills. Although graduate students are well versed in their specialties, few have ever presented material to young students; the DNALC supports them as they do.

BioMedia Visitation and Projects

We again had a record number of visitors to our suite of multimedia resources in 2015. Google Analytics counted 5,460,083 visits to 24 DNALC websites. YouTube videos received 881,079 views, and the 3D Brain, Weed to Wonder, and Gene Screen apps were downloaded 1,186,319 times. Total website, YouTube, and smartphone/tablet app visitation reached 7.52 million, an 11% increase over 2014. In-app purchases of 3D Brain HQ netted nearly $10,000 in 2015. We worked with the Einstein Victor Center for Jewish Genetic Diseases to give our Gene Screen app an interface facelift and to update it to fit on all iOS device sizes. Gene Screen was very popular in 2015 with 413,107 downloads, more than four times the prior year.

The BioMedia Group supports the DNALC’s educational programs with website development and maintenance; videography and post production; program promotion through email campaigns; flyer, poster, and backdrop design; museum development; photo documentation of events and barcoding specimens; and social media management. 2015 saw an increase in development of architectural concept plans for DNALC venues and institutions adding DNALC-style laboratory classrooms. Our concept plans are often the first visualization of a potential lab or new DNALC licensed venue; this year we worked on the following:

- **DNALC Asia:** DNALC concept plans were used to solicit design and construction bids prior to renovation that started in October. The plan included eight teaching labs, two bioinformatics labs, prep labs, and offices in a two-story circular building, as well as an exhibition hall in an all-glass dome building and a separate cafeteria, all covering more than 30,000 square feet. We also worked closely with Centerbrook Architects to develop a 3D concept plan of the interior of a cell displayed in the all-glass dome building; the plan was proposed to the SIP Advisory Committee at a visit to CSHL in July.

- **Potential DNALC at CUNY in Brooklyn:** We created a plan for about 25,000 square feet of space. Centerbrook Architects and Planners then designed and created a 3D rendering with four teaching labs, two research labs, two bioinformatics classrooms, offices, lunchroom, and exhibition space.

- **Beijing 166 High School:** Two laboratory classrooms were created to accommodate up to 45 students.

- **Godfrey Okoye University, Nigeria:** The proposed plan included three DNALC signature teaching labs, research lab, prep lab, bioinformatics lab, 1000-square-foot exhibition gallery, conference room, and office space.

- **North Royalton High School, winner of the Breakthrough Junior Challenge Prize:** We are working with school staff to help develop a DNALC signature teaching lab and prep areas adjacent to typical classroom space.

BOLD (named for the acronym for the Barcode of Life Data Systems), an art exhibition by Seattle-based artist Joseph Rossano, was installed in the front and side halls of the DNALC in September 2014. For the artwork in BOLD, Rossano partnered with biologists to engage the public around the science of DNA barcoding and how it is being used to catalog the world’s vast and threatened biodiversity. During the year, the BioMedia Group developed a BOLD website to provide a deeper understanding of the species featured in the artwork; the site is directly linked
through QR codes on the labels adjacent to each work (http://museum.dnalc.org/bold/). On the site, Rossano provides a video introduction to each series in the show, and viewers can link to the BOLD database entry for the organism depicted in the art. We also worked with DNALC educators to develop a student guide for the art exhibit and collaborated with Rossano, researchers, and art critics to develop a catalog booklet for the show.

In September, we hosted an event for BOLD. Guests began the evening viewing the artwork at a reception at the DNALC, and then moved to Grace Auditorium on the CSHL campus for a talk by Dr. Daniel Janzen from the Área de Conservación Guanacaste (ACG) in Costa Rica and Professor of Biology at the University of Pennsylvania. Janzen’s work, as well as that of researchers from the University of California, Berkeley, Moorea Biocode Project at the Richard B. Gump South Pacific Research Station in Moorea, served as inspiration for Rossano’s art. Janzen’s talk, “Exploring the world from its insides out, as well as outsides in; how to really save tropical biodiversity,” focused on how he employs local parataxonomists in Costa Rica to aid in his conservation and research efforts in ACG.

The BioMedia Group played a significant role in the development of the Ötzi display. We cooperated with the CSHL facilities department to transform the exhibit space into the Italian Alps over the summer, including a full-wall mural of the Ötztal Mountains where the mummy was found in 1991. A majority of the space was completed in time to welcome a NOVA film crew working on the Iceman Reborn documentary being produced by BSquared Media. The crew filmed sculptor Gary Staab with the Ötzi replica and fielded questions with students attending our summer camps (see photo); interviewed Dr. Watson, director Dave Micklos, and educator Elna Carrasco; and shot lots of “B-roll” footage at the DNALC and on the CSHL campus. Through the fall, content for the interpretive labels was developed working with DNALC educators, an all-glass case arrived to house Ötzi, and finishing touches were put on the exhibit space. NOVA Iceman Reborn will air on PBS in February 2016.
Staff and Interns

In 2015, we said goodbye to several staff members. In January, Mohammed Khalfan accepted a position as a Bioinformatics Specialist at the Center for Genomics and Systems Biology at New York University. He joined the DNALC as a computer programmer in 2010 and worked on many projects, including DNA Subway.

Brooke Roeper Hohman began with our teaching team in 2012. With a background in education, she was a perfect fit to our instructional family, teaching mostly high school and some middle school classes. Shortly after her wedding in the spring, she accepted a position at Evolution Health as manager of Outcomes and Epidemiology, which relocated her to her roots in Ohio.

Antonia Florio began with us in 2013, as a Conservation Genetics instructor, and was promoted to Manager, Research in 2014. She assumed the assessment of project proposals, arranging for the evaluations, planning/organizing project symposiums, and lab instruction at all three DNALC facilities. She was also responsible for applying for funding, facilitating student evaluation, and guiding new staff heading BLI. In August, she left the DNALC for a full-time professorship at Saint Francis College in Brooklyn and is teaching Introduction to Cell Biology and Forensics.

Hired as a college intern in 2013, Ashleigh Jackobel was promoted to Lab Instructor the following year. Her enthusiasm and skill allowed her to make the transition from lab to classroom flawlessly. She was adept at teaching both middle and high school classes, helped with lab prep, and worked with interns. In the fall, she left to pursue a graduate degree at SUNY Upstate Medical University in Syracuse, New York.

A technician in Greg Hannon’s lab, Emily Harrison became a part-time Middle School Instructor in 2014. While honing her skills as a teacher, she applied and was accepted into the University of California Berkeley’s Science Education Ph.D. program.

Shreya Shah began in 2014 as a high school educator and an assistant manager for BLI. When her husband was hired for a teaching position at Westchester University in Pennsylvania, she had the opportunity to join him there as adjunct faculty in the summer.

With several positions to fill, two educators joined the DNALC in June. Sharon Pepenella stepped in as a High School Educator after more than a decade studying in western New York. She completed a Bachelor of Science from SUNY Geneseo, moving on to earn a Ph.D. in biochemistry from the University of Rochester. Her research focused on the histone protein H4 tail domain and its contributions to the formation of chromatin structure. Her teaching experience includes hands-on science education with elementary school students, as well as mentoring and tutoring undergraduate and graduate students. Sharon is co-managing Barcode Long Island and also oversees laboratory prep and troubleshooting.

Cristina Fernandez–Marco of Santander, Spain, joined the DNALC as a high school educator after studying the molecular genetics of plant development on the CSHL campus. Before coming to the United States, Cristina received her Ph.D. in Plant Biology from the Spanish National Research Council. Cristina co-manages BLI and is also developing labs for student research programs.

Paul Donat, a college intern since 2012, was hired as our first full-time lab technician in 2015. After graduating SUNY Geneseo with a B.S. in Biochemistry, he moved back to Long Island just as we found ourselves requiring more help in the lab, so we created a new position for him. Paul assisted with training college interns and organizing prep for all of our onsite and offsite courses, international collaborations, teacher trainings, BLI open labs and footlockers, and he developed a new organization system for our equipment. In June, Paul was promoted to full-time high school educator.

Emtiaz Uddin started in June, replacing Paul as lab technician. A recent graduate of Stony Brook University with a Bachelor’s degree in Biology, he is currently working on a Master’s Degree in Health Administration at Hofstra University. Emtiaz was born and raised in Elmont, New York,
and enhanced his experience in the sciences through an internship with the North Shore–Long Island Jewish medical system.

Pan Teng joined the DNALC as a bioinformatician. Originally from Xi’an, Shaanxi, China, she completed a Master of Science degree in Bioinformatics at the University of Delaware. Her role as a Research Assistant exploring cellular function at the University of Delaware strengthened her knowledge in the field of bioinformatics, making her a valuable addition to the team at the DNALC. Pan helps build and maintain the DNALC websites and develops software.

After more than a year without a Program Evaluator, we were thrilled to welcome Lindsay Barone of Oregon, Wisconsin, in November. She received a Master of Science and a Ph.D. in Anthropology from the University of Wisconsin, Milwaukee. She also earned minor degrees in museum studies and biological sciences. Before joining the DNALC, Lindsay worked as an adjunct professor, lab instructor, and intern at several institutions, including the American Museum of Natural History. Lindsay will be evaluating several projects at the DNALC, including RNA-Seq for the Next Generation, iPlant, and DNALC collaborations in China.

Joslynn Lee started in a new position, Data Science Educator, just after Thanksgiving. Originally from Upper Fruitland, New Mexico, Joslynn completed a Bachelor of Science in Chemistry at Fort Lewis College in Durango, Colorado, and became a research associate in bioanalytical chemistry for Vertex Pharmaceuticals, Inc., in Cambridge, Massachusetts. She went on to pursue a Ph.D. in Chemistry from Northeastern University in Boston. Joslynn joins the DNALC after completing her postdoctoral research at the University of Minnesota Medical School in Duluth. She will develop course content and lead training workshops on bioinformatics and data science for iPlant Collaborative.

Since the DNALC opened, we have relied on high school and college interns to support our day-to-day operations. The BioMedia Group also welcomes interns for summer or longer-term roles. An internship offers students the unique opportunity to gain real laboratory or design experience in an educational environment. We gathered an amazing group of interns this year and said farewell as others left for college.

### High School Interns

<table>
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<tr>
<th>Brady Anna, St. Anthony’s High School</th>
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<tr>
<td>Alyssa DiArrigo, Bethpage High School</td>
<td>Rahul Ranjan, Hicksville High School</td>
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<td>Juliana Eastment, Oyster Bay High School</td>
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<td>Alec Haber, Syosset High School</td>
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<td>John Messina, Chaminade High School</td>
<td>Bijia Wang, Syosset High School</td>
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<td>Stefanie Montalbano, Our Lady of Mercy Academy</td>
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<td>Gabrielle Nagel, Friends Academy</td>
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High School Interns Departing for College

Kayla Bianco, Bucknell University
Abigail Buckley, Dartmouth College
Kenneth Collado, Stony Brook University
Liam Gensel, State University of New York, Buffalo
Omotayo Ikuomenisan, Hunter College
Aiseosa Irowa
Lenni Joya Amaya, Marist College

College Interns

Kathryn Bellissimo, The College of New Jersey
Gabrielle Blazich, Fordham University
Brittany Coscio, College of the Holy Cross
Ariana Forohar, University of California, Los Angeles
Nella Hauser, Stony Brook University
Marie Jean Francois, CUNY City College

Sites of Major Faculty Workshops

Program Key: 

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<td>Laney College, Oakland</td>
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<td>Lutheran University, Thousand Oaks</td>
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<td>Oxnard Community College, Oxnard</td>
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<td>Pierce College, Los Angeles</td>
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<td>Salk Institute for Biological Studies, La Jolla</td>
<td>2001, 2008</td>
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<td>Southwestern College, Chula Vista</td>
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<td>Stanford University, Palo Alto</td>
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<td>University of California, Berkeley</td>
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University of California, Davis 1986
University of California, Davis 2012, 2014, 2015
University of California, Long Beach 2015
University of California, Northridge 1993
University of California, Riverside 2011
University of California, Riverside 2012
University of California, San Francisco 2015

COLORADO
Aspen Science Center 2006
Colorado State University, Fort Collins 2013
Community College of Denver 2014
United States Air Force Academy, Colorado Springs 1995
University of Colorado, Denver 1998, 2009–2010
Choate Rosemary Hall, Wallingford 1987

CONNECTICUT

DISTRICT OF COLUMBIA

FLORIDA
Armwood Senior High School, Tampa 1991
Florida Agricultural & Mechanical University, Tallahassee 2007–2008
Florida Agricultural & Mechanical University, Tallahassee 2011
Florida SouthWestern State University, Fort Myers 2015
North Miami Beach Senior High School 1991
Seminole State College, Sanford 2013–2014
University of Miami School of Medicine 2000
University of Western Florida, Pensacola 1991
Fernbank Science Center, Atlanta 1989, 2007

GEORGIA
Spelman College, Atlanta 2010

HAWAII
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
Kings College, Chicago 2014

INDIANA
Purdue University, West Lafayette 2012

IDAHO
University of Idaho, Moscow 1994

IOWA
Drake University, Des Moines 1987

KANSAS
University of Kansas, Lawrence 1995

KENTUCKY
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

MARYLAND
Bowie State University 2011, 2015
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
<p>| MASSACHUSETTS | Arnold Arboretum of Harvard University, Roslindale | 2011 |
|              | Beverly High School                             | 1986 |
|              | Biogen Idec, Cambridge                          | 2002, 2010 |
|              | <strong>Boston University</strong>                            | <strong>1994, 1996</strong> |
|              | CityLab, Boston University School of Medicine    | 1997 |
|              | Dover-Sherborn High School, Dover                | 1989 |
|              | Randolph High School                            | 1988 |
|              | The Winsor School, Boston                        | 1987 |
|              | Whitehead Institute for Biomedical Research, Cambridge | 2002 |
| MICHIGAN     | Athens High School, Troy                        | 1989 |
| MINNESOTA    | Schoolcraft College, Livonia                    | 2012 |
|              | <strong>American Society of Plant Biologists, Minneapolis</strong> | 2015 |
|              | Minneapolis Community and Technical College, Madison | 2009 |
|              | <strong>Minneapolis Community and Technical College, Madison</strong> | 2013 |
|              | University of Minnesota, St. Paul               | 2005 |
| MISSISSIPPI  | <strong>University of Minnesota, St. Paul</strong>           | <strong>2010</strong> |
| MISSOURI     | Mississippi School for Math &amp; Science, Columbus | 1990–1991 |
| MONTANA      | Montana State University, Bozeman               | 2012 |
| NEBRASKA     | University of Nebraska-Lincoln, Lincoln         | 2014 |
| NEVADA       | University of Nevada, Reno                      | 1992, 2014 |
| NEW HAMPSHIRE| Great Bay Community College, Portsmouth         | 2009 |
|              | <strong>New Hampshire Community Technical College, Portsmouth</strong> | 1999 |
|              | St. Paul’s School, Concord                       | 1986–1987 |
| NEW JERSEY   | Coriell Institute for Medical Research, Camden  | 2003 |
|              | Kean University, Union Township                  | 2003 |
|              | <strong>Cold Spring Harbor Laboratory</strong>               | <strong>2014, 2015</strong> |
|              | Columbia University, New York                    | 1993 |
|              | <strong>Cornell University, Ithaca</strong>                  | <strong>2005</strong> |
|              | DeWitt Middle School, Ithaca                    | 1991, 1993 |
| NEW YORK     | Albany High School                              | 1987 |
|              | Bronx High School of Science                   | 1987 |
|              | Brookhaven National Laboratory, Upton           | 2015 |
|              | Canisius College, Buffalo                      | 2007 |
|              | City College of New York                        | 2012 |
|              | Cold Spring Harbor High School                  | 1985, 1987 |
|              | <strong>Cold Spring Harbor Laboratory</strong>               | <strong>2014, 2015</strong> |
|              | Columbia University, New York                   | 1993 |
|              | Cornell University, Ithaca                      | 2005 |
|              | DeWitt Middle School, Ithaca                    | 1991, 1993 |
|              | <strong>Dolan DNA Learning Center</strong>                   | <strong>1990–1992</strong> |
|              | DNA Learning Center West                        | 2005 |
|              | Environmental Science Center, Bergen Beach, Brooklyn | 2015 |
|              | Fostertown School, Newburgh                     | 1991 |
|              | <strong>Harlem DNA Lab, East Harlem</strong>                 | <strong>2015</strong> |
|              | 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 |</p>
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<td>Nassau Community College, Garden City</td>
<td>2013</td>
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<td>New York Botanical Garden, Bronx</td>
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<td>New York City Department of Education</td>
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<td>New York Institute of Technology, New York</td>
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<td>Orchard Park Junior High School</td>
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<td>Plainview-Old Bethpage Middle School</td>
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<td>State University of New York, Purchase</td>
<td>1989</td>
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<td>State University of New York, Stony Brook</td>
<td>2014</td>
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<tr>
<td>The Rockefeller University, New York</td>
<td>2003, 2015</td>
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<td>Titusville Middle School, Poughkeepsie</td>
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<td>Trudeau Institute, Saranac Lake</td>
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<td>Union College, Schenectady</td>
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<td>United States Military Academy, West Point</td>
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<td>Wheatley School, Old Westbury</td>
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<td>CIIT Center for Health Research, Triangle Park</td>
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<td>North Carolina School of Science, Durham</td>
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<td>North Carolina State University, Raleigh</td>
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<td>North Dakota State University, Fargo</td>
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<td>Case Western Reserve University, Cleveland</td>
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<td>Cleveland Clinic</td>
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<td>Langston University, Langston</td>
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<td>North Westerville High School</td>
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<td>Oklahoma City Community College</td>
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<td>Oklahoma Medical Research Foundation, Oklahoma City</td>
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<td>Oklahoma School of Science and Math, Oklahoma City</td>
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<td>Tulsa Community College, Tulsa</td>
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<td>Kaiser Permanente–Center for Health Research, Portland</td>
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<td>Linfield College, McMinnville</td>
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<td>Duquesne University, Pittsburgh</td>
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<td>Germantown Academy</td>
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<td>Kimmel Cancer Center, Philadelphia</td>
<td>2008</td>
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<td>Botanical Society of America, Providence</td>
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<td>Clemson University</td>
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<td>Medical University of South Carolina, Charleston</td>
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<td>University of South Carolina, Columbia</td>
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<td>South Dakota State University, Brookings</td>
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<td>University of South Carolina, Columbia</td>
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<td>University of Lone Star College, Kingwood</td>
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<td>Midland College</td>
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<td>Southwest Foundation for Biomedical Research, San Antonio</td>
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<td>Texas A&amp;M University, College Station</td>
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<td>Texas A&amp;M University, Prairie View</td>
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<td>Texas A&amp;M University, AG Research and Extension Center, Weslaco</td>
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<td>Trinity University, San Antonio</td>
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University of Texas, Brownsville 2010
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

University of Vermont, Burlington 1989

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

Shoreline Community College 2011, 2012


Bethany College 1989

Blood Center of Southeastern Wisconsin, Milwaukee 2003

Marquette University, Milwaukee 1986–1987
University of Wisconsin, Madison 1988–1989

University of Wisconsin, Madison 2004, 2012

University of Wyoming, Laramie 1991

University of Puerto Rico, Mayaguez 1992
University of Puerto Rico, Rio Piedras 1993
University of Puerto Rico, Rio Piedras 1994

Walter and Eliza Hall Institute and University of Melbourne 1996
Vienna Open Lab, Vienna 2007, 2012
Red River Community College, Winnipeg, Manitoba 1989
Beijing No. 166 High School, Beijing 2013, 2014, 2015
Ho Yu College, Hong Kong 2009

Faroe Genome Project, Torshavn, Faroe Islands 2013
Urania Science Center, Berlin 2008

European Conference on Computational Biology/Intelligent System for Molecular Biology Conference, Dublin 2015

International Institute of Genetics and Biophysics, Naples 1996
Porto Conte Research and Training Laboratories, Alghero 1993

ASPB Plant Biology, Merida 2008
Godfrey Okoye University, Enugu, Nigeria, Africa 2013

University of Panama, Panama City 1994

Shemyakin Institute of Bioorganic Chemistry, Moscow 1991

National Institute of Education 2001–2005

Kristineberg Marine Research Station, Fiskebackskil 1995
Uppsala University 2000

International Chromosome Conference, Amsterdam 2007

Wageningen University and Research Center, Wageningen 2014

The Genome Analysis Center, Norwich 2015
Wellcome Trust Conference Center, Hinxton 2012–2013
University of Warwick, Coventry 2013
Workshops, Meetings, Collaborations, and Site Visits

January 5–9     NSF ATE Professional Development *Genomic Approaches in BioSciences* Workshop, Southwestern College, Chula Vista, California

Jan 6–Feb 12    Pinkerton *Urban Barcode Research Project, Conservation Genetics* Workshop (Tuesdays and Thursdays), American Museum of Natural History, New York

January 10      Saturday DNA! “The Eyes Have It,” DNALC

January 1–4     International Plant and Animal Genome XXIII Conference 2015, *Bioinformatic Ice Cream Social*, San Diego, California

January 13      *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab

January 15      NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC

January 15      *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab

January 22      Site visit by Dr. J. Armando Barriguete and Alonso Sanz, Mexico City, Mexico

January 22      Site visit by Dr. Ben Dubin-Thaler, Harlem DNA Lab

January 29–30   NSF iPlant Collaborative Professional Development *Tools & Services* Workshop, Clemson University, Clemson, South Carolina

February 2      Center for Education Innovation—Public Education Association Professional Development Short DNA Fingerprinting Workshop, Harlem DNA Lab

February 2–6    *DNA Science Workshops* with students from Beijing No. 166 School, Beijing, China, CSHL

February 3      *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab

February 3      CSHL Gramene: *A Resource for Comparative Plant Genomics* Webinar, DNALC

February 6      Site visit by Lilly Wang and Jun Go, State University of New York, Albany

February 6      NSF Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Video Conference, DNALC

February 7      Saturday DNA! “Gram-tastic!” DNALC

February 9–13   *DNA Barcoding Workshops* with students from Beijing No. 166 School, Beijing, China, CSHL

February 11     NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC

February 12     *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab


February 24     *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab

February 24     CSHL Gramene: *A Resource for Comparative Plant Genomics* Webinar, DNALC


March 5         *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab

March 6–8       NSF iPlant Collaborative Software Carpentry Workshop, New York Academy of Science, New York

March 8–14      *DNA Barcoding Workshop*, Environmental Science Center, Bergen Beach, Brooklyn, New York

March 10        *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab

March 10        NIH Barcode Long Island Presentation, “A Day in the Life…,” Brookhaven National Laboratory, Upton, New York

March 11        NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC

March 12        *DNA Barcoding Presentation*, Bronx Community College, Bronx, New York

March 14        Saturday DNA! “Cauliflower Cloning,” DNALC

March 14        NIH Barcode Long Island Workshop, DNALC

March 17        CSHL Gramene: *A Resource for Comparative Plant Genomics* Webinar, DNALC

March 19        *Urban Barcode Project/Urban Barcode Research Project* Open Lab, Harlem DNA Lab


March 21        NIH Barcode Long Island Workshop, DNALC

March 23–26     NSF iPlant Collaborative Data Carpentry Hack-a-thon Workshop, CSHL

March 23–27     NSF ATE Professional Development *Genomic Approaches in BioSciences* Workshop, University of California, San Francisco

March 24        NIH Barcode Long Island Open Lab, DNALC

March 25        Site visit by Dr. Diego Ulibarri, A.D.N. Mexico, Morelia, Mexico

March 25–27     Site visit by Andrea Jimenez Garcia, Mexico City, Mexico


March 30        Site visit by David Manning, Scott Bronson, and Ken White, Brookhaven National Laboratory, Upton, New York

April 6–10      Pinkerton *Urban Barcode Research Project, DNA Barcoding Workshop*, Rockefeller University, New York

April 7         CSHL Gramene: *A Resource for Comparative Plant Genomics* Webinar, DNALC

April 8         NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC

April 10  Site visit by Bill Schierberl with Ivory Coast Education Committee, Université de Cocody, Abidjan, Ivory Coast, Africa

April 13–15  DNA Learning Center Fingerprinting Workshops, with students from Beijing No. 166 School, Beijing, China

April 14  NSF Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Video Conference, DNA Learning Center

April 16  Site visit by Paul Orselli, Paul Orselli Workshop, Baldwin, New York

April 16  NIH Barcode Long Island Open Lab, DNA Learning Center

April 16  Math for America Learning Center, DNA Barcoding Information Session, New York

April 17  Site visit by Russell Holtzer, Gillian Small, Alan Dobrin, and Judy Bergtraum, City University of New York, New York

April 17–22  Fingerprinting Workshops at Beijing 166 School, Beijing, China DNA Learning Center

April 18  NIH Barcode Long Island Workshop, DNA Learning Center


April 25  Saturday DNA! “Bacteria and Antibiotics,” DNA Learning Center

April 25  NIH Barcode Long Island Workshop, DNA Learning Center

April 27  Site visit by Yaqin Xu, Weill Cornell Medical College, New York


May 1  Site visit by Dr. Julian Rayner, Wellcome Trust Genome Campus, Hinxton, England

May 2  NIH Barcode Long Island Workshop, DNA Learning Center

May 4  NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNA Learning Center


May 7–8  NSF iPlant Tools & Services Workshop, University of Georgia, Athens, Georgia

May 9  NIH Barcode Long Island Workshop, Stony Brook University, Stony Brook, New York


May 14  “Professor for a Day,” Yeshiva University, Cold Spring Harbor Laboratory

May 15  Site visit by Dr. Ben Dubin-Thaler, BioBus, New York

May 16  Saturday DNA! “The Genetic Engineering Toolbox,” DNA Learning Center

May 18–19  NSF iPlant Collaborative Tools & Services Workshop, Santa Fe Community College, Santa Fe, New Mexico

May 19  Cold Spring Harbor Laboratory, DNA Learning Center

May 19  Site visit by Instructional Group, Lycee Francais, New York

May 21–22  NSF iPlant Collaborative Genomics in Education Workshop, Santa Fe Community College, Santa Fe, New Mexico


May 27  Urban Barcode Project Poster Session, American Museum of Natural History, New York

May 28–29  American Society for Microbiology Conference for Undergraduate Education, “The Urban Barcode Research Project,” Austin, Texas

May 29  NSF Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Webinar, DNA Learning Center


June 3  NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNA Learning Center

June 3  NSF Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Video Conference, DNA Learning Center

June 4  Center for Education Innovation—Public Education Association Professional Development Workshop, “Restriction Analysis,” Harlem DNA Lab

June 5  NIH Barcode Long Island Symposium, DNA Learning Center

June 6  Cold Spring Harbor Laboratory, DNA Learning Center

June 6  125th Anniversary Open House, “DNA Extraction & Observing Mutant Organisms,” Cold Spring Harbor Laboratory

June 6  Saturday DNA! “Going the Distance!” DNA Learning Center

June 6  The New York City Biome Map Bacterial Printing Workshop, Genspace, Brooklyn, New York

June 8  Urban Barcode Project Symposium, American Museum of Natural History, New York


June 8–12  NSF Professional Development Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Workshop, Bowie State University, Bowie, Maryland

June 8–12  Fun with DNA Workshop, Convent of the Sacred Heart, Greenwich, Connecticut
<table>
<thead>
<tr>
<th>Date</th>
<th>Event Description</th>
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<tr>
<td>June 15</td>
<td>Pinkerton Urban Barcode Research Project Symposium, American Museum of Natural History, New York</td>
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<tr>
<td>June 15−16</td>
<td>NSF iPlant Tools &amp; Services Workshop, South Dakota State University, Brookings</td>
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<tr>
<td>June 15−19</td>
<td>Forensics Workshop, St. David’s School, New York</td>
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<tr>
<td>June 15−19</td>
<td>World of Enzymes Workshop, Convent of the Sacred Heart, Greenwich, Connecticut</td>
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<tr>
<td>June 18−19</td>
<td>NSF iPlant Genomics in Education Workshop, South Dakota State University, Brookings</td>
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<tr>
<td>June 22</td>
<td>NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC</td>
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<tr>
<td>June 22−26</td>
<td>CSHL Gramene: A Resource for Comparative Plant Genomics Webinar, DNALC</td>
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<tr>
<td>June 22−26</td>
<td>Fun with DNA Workshop, Marymount School, New York</td>
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<tr>
<td>June 22−26</td>
<td>NSF Professional Development Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Workshop, California State University, Long Beach</td>
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<td>June 29−July 2</td>
<td>Forensic Detectives Workshop, DNALC</td>
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<tr>
<td>June 29−July 2</td>
<td>Fun with DNA Workshop, DNALC</td>
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<td>June 29−July 2</td>
<td>Green Genes Workshop, DNALC</td>
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<td>June 29−July 17</td>
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<td>Fun with DNA Workshop, DNALC</td>
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<td>June 30</td>
<td>NSF iPlant Collaborative Webinar</td>
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<td>July 2−3</td>
<td>NSF iPlant Collaborative Tools &amp; Services Workshop, The Genome Analysis Center, Norwich, United Kingdom</td>
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<td>DNA Science Workshop, DNALC</td>
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<td>DNA Barcoding Workshop, Harlem DNA Lab</td>
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<td>July 7−8</td>
<td>Lightning Talk: “NSF iPlant Collaborative Resources for Science,” Galaxy Community Conference, Norwich, United Kingdom</td>
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<td>July 13−17</td>
<td>Forensic Detectives Workshop, DNALC</td>
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<td>Fun with DNA/World of Enzymes Workshop with students from Beijing No. 166 School, China DNALC</td>
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<td>Genome Science Workshop, DNALC</td>
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<td>DNA Science Workshop, Harlem DNA Lab</td>
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<td>July 14</td>
<td>CSHL Gramene: A Resource for Comparative Plant Genomics Webinar, DNALC</td>
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<td>July 15</td>
<td>NIH Barcode Long Island Workshop, Brookhaven National Laboratory, Upton, New York</td>
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<td>July 22</td>
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<td>July 26−30</td>
<td>American Society of Plant Biologists Plant Biology 2015 Conference, Minneapolis, Minnesota</td>
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<td>Pinkerton Urban Barcode Research Project DNA Barcoding Workshop, Harlem DNA Lab</td>
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<tr>
<td>July 27−31</td>
<td>Forensics Workshop with students from Beijing No. 166 School, China DNALC</td>
</tr>
<tr>
<td>July 27−31</td>
<td>Green Genes Workshop, DNALC (two sessions)</td>
</tr>
<tr>
<td>July 27−31</td>
<td>Fun with DNA Workshop, DNALC West</td>
</tr>
<tr>
<td>August 3−6</td>
<td>Pinkerton Urban Barcode Research Project Conservation Genetics Workshop, Harlem DNA Lab</td>
</tr>
<tr>
<td>August 3−7</td>
<td>NIH Barcode Long Island Workshop, Stony Brook University, Stony Brook, New York</td>
</tr>
<tr>
<td>August 3−7</td>
<td>Fun with DNA Workshop, DNALC</td>
</tr>
<tr>
<td>August 3−7</td>
<td>DNA Science Workshop, DNALC West</td>
</tr>
<tr>
<td>August 3−7, 10−14</td>
<td>DNA Barcoding Workshops, Environmental Study Center, Brooklyn, New York</td>
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<tr>
<td>August 10−14</td>
<td>NIH Barcode Long Island Workshop, Brookhaven National Laboratory, Upton, New York</td>
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<tr>
<td>August 10−14</td>
<td>DNA Science Workshop, DNALC</td>
</tr>
<tr>
<td>August 10−14</td>
<td>Fun with DNA Workshop, DNALC (two sessions)</td>
</tr>
<tr>
<td>August 10−14</td>
<td>Backyard Barcoding Workshop, DNALC West</td>
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</tbody>
</table>
August 11  NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC
August 11−12  Urban Barcode Project DNA Barcoding Teacher Training Workshop, Harlem DNA Lab
August 17  NSF Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Webinar, DNALC
August 17−21  Fun with DNA Workshop, DNALC
Green Genes Workshop, DNALC
Silencing Genomes Workshop, DNALC
World of Enzymes Workshop, DNALC
DNA Science Workshop, Beckman Laboratory, CSHL
Genome Science Workshop, DNALC West
August 18  NSF iPlant Collaborative United States Department of Agriculture/Agricultural Research Service Workshop, University of Georgia, Athens
August 18  CSHL Gramene: A Resource for Comparative Plant Genomics Webinar, DNALC
August 19−20  NSF iPlant Collaborative Genomics in Education Workshop, Florida SouthWestern State University, Fort Myers, Florida
August 20−21  DNA Barcoding Teacher Training Workshop, Harlem DNA Lab
September 4  World Science Festival 2015, “The DNALC” Lecture, Vienna, Austria
September 8  Vienna Open Lab and Science Centre Network, Vienna, Austria
September 9  NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC
September 18  The Icahn School of Medicine at Mount Sinai Science Education & Outreach Post-doc Symposium Presentation, “The DNALC,” The Icahn School of Medicine at Mount Sinai, New York
September 21−23  NSF iPlant Tools & Services Workshop, University of California, Davis
September 25  BOLD Art Exhibit Reception and Daniel Janzen talk, DNALC and CSHL
October 7  NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC
October 9  NSF Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Webinar, DNALC
October 13−15  Introduction to Genetics and Biotechnology Workshop, Beijing No. 166 School, Beijing, China
October 17  Saturday DNA! “Biological Evidence,” DNALC
October 17−21  DNA Barcoding Workshops with students from Beijing No. 166 School, Beijing, China
October 20  CSHL Gramene: A Resource for Comparative Plant Genomics Webinar, DNALC
October 24  NIH Barcode Long Island Open Lab, Stony Brook University by Dan Maloney, Stony Brook, New York
October 24  NIH Barcode Long Island Open Lab, DNALC West
October 27  Simons Foundation Math and Science Education Workshop, New York
November 3  NIH Barcode Long Island Workshop, DNALC
November 3  NIH Barcode Long Island Information Session, Brookhaven National Laboratory, Upton, New York
November 3  Center for Education Innovation—Public Education Association Professional Development Workshop, Bacterial Transformation Lab, Harlem DNA Lab
November 4  NSF iPlant Collaborative Webinar, “Getting Started with iPlant,” DNALC
November 7  Saturday DNA! “Seeds of Success,” DNALC
November 7  NIH Barcode Long Island Open Lab, Stony Brook University, Stony Brook, New York
November 12  Regeneron Pharmaceuticals Inc. Celebration, Tarrytown, New York
November 14  NIH Barcode Long Island Open Lab, DNALC
November 17  CSHL Gramene: A Resource for Comparative Plant Genomics Webinar, DNALC
November 18  NIH Barcode Long Island Open Lab, DNALC West
November 18, 19  NSF Infrastructure and Training Bringing Next-Generation Sequence (NGS) Analysis into Undergraduate Education Video Conference, DNALC
November 19  Site visit by Hala Mirza, Potoula Gjidia and Representative Team, Regeneron Pharmaceuticals Inc., Tarrytown, New York, DNALC West
November 19  Award presentation to Christine Marizzi, AgarArt Gallery event, American Society of Microbiology, Washington, D.C.
November 21  NIH Barcode Long Island Open Lab, DNALC
December 1  NIH Barcode Long Island Open Lab, DNALC
December 2  NSF iPlant Collaborative Genome Assembly, Annotation, and RNA-Seq Half-Day Workshop, CSHL
December 2  Pinkerton Urban Barcode Research Project Open Lab, American Museum of Natural History, New York
December 5  Saturday DNA! “The Iceman Cometh,” DNALC
December 5  Urban Barcode Project/Urban Barcode Research Project Open Lab, Harlem DNA Lab
December 5  NIH Barcode Long Island Open Lab, Stony Brook University, Stony Brook, New York
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<tr>
<th>Date</th>
<th>Event</th>
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<tr>
<td>December 5</td>
<td>Commencement, Godfrey Okoye University, Enugu, Nigeria</td>
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<td>December 8</td>
<td><em>Urban Barcode Project/Urban Barcode Research Project</em> Open Lab, <em>Harlem DNA Lab</em></td>
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<td>December 9</td>
<td>Site Visit by Parker Antin, <em>iPlant Collaborative</em>, University of Arizona</td>
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<td>December 12</td>
<td><em>Urban Barcode Research Project</em> activities, Irondale Theatre Company, Brooklyn, New York</td>
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<td>December 12</td>
<td>NIH <em>Barcode Long Island</em> Open Lab, DNALC</td>
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<tr>
<td>December 14</td>
<td>Site Visit by Dr. Russel Hotzler, Gustavo Ordonez, Meghan Mulgrew, City University of New York, New York</td>
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<tr>
<td>December 15</td>
<td>NIH <em>Barcode Long Island</em> Open Lab, Brookhaven National Laboratory, Upton, New York</td>
</tr>
<tr>
<td>December 15</td>
<td>NSF <em>iPlant Collaborative</em> Webinar, “Getting Started with iPlant,” DNALC</td>
</tr>
<tr>
<td>December 15</td>
<td>CSHL <em>Gramene: A Resource for Comparative Plant Genomics</em> Webinar, DNALC</td>
</tr>
<tr>
<td>December 15−23</td>
<td>Site visit by Dorothy Zhang, CSHL Asia and DNALC Asia, Suzhou, China</td>
</tr>
<tr>
<td>December 16</td>
<td>NIH <em>Barcode Long Island</em> Open Lab hosted by Aleida Perez, Brookhaven National Laboratory, Upton, New York</td>
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<tr>
<td>December 18</td>
<td>Site visit by Kristina and Peter Liu, WI Harper Group, San Francisco, California</td>
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</table>
PRESS PUBLICATIONS

Serials

- Genes & Development, Vol. 29 (www.genesdev.org)
- Genome Research, Vol. 25 (www.genome.org)
- Learning & Memory, Vol. 22 (www.learnmem.org)
- RNA, Vol. 21 (www.rnajournal.org)
- Cold Spring Harbor Symposia in Quantitative Biology, Vol. 79: Cognition, edited by Cori Bargmann, Daphne Bavelier, Terrence Sejnowski, David Stewart, and Bruce Stillman
- Cold Spring Harbor Perspectives in Biology (www.cshperspectives.org)
- Cold Spring Harbor Perspectives in Medicine (www.perspectivesinmedicine.org)
- Cold Spring Harbor Molecular Case Studies (www.molecularcasestudies.org)

Laboratory Manuals

- Cell Death Techniques: A Laboratory Manual, edited by Ricky W. Johnstone and John Silke

Handbooks

- Using R at the Bench: Step-by-Step Data Analytics for Biologists, by Martina Bremer and Rebecca W. Doerge

Textbooks

- Epigenetics, Second Edition, edited by C. David Allis, Marie-Laure Caparros, Thomas Jenuwein, and Danny Reinberg; Associate Editor Monika Lachlan

Monographs (Topic Collections from Perspectives in Biology and Perspectives in Medicine)

- Retinal Disorders: Genetic Approaches to Diagnosis and Treatment, edited by Eric A. Pierce, Richard H. Masland, and Joan W. Miller
- Glia, edited by Ben A. Barres, Marc R. Freeman, and Beth Stevens
- Intellectual Property in Molecular Medicine, edited by Salim Mamajiwalla and Rochelle Seide
- Mitosis, edited by Mitsuhiro Yanagida, Anthony A. Hyman, and Jonathon Pines

E-books (Kindle Editions)

- Epigenetics, Second Edition, edited by C. David Allis, Marie-Laure Caparros, Thomas Jenuwein, and Danny Reinberg; Associate Editor Monika Lachlan
- Orphan: The Quest to Save Children with Rare Genetic Disorders, by Philip R. Reilly
- Decoding the Language of Genetics, by David Botstein
- CSHL Annual Report 2014, Yearbook Edition
- Banbury Center Annual Report 2014

History

- The Road to Discovery: A Short History of Cold Spring Harbor Laboratory, by Jan A. Witkowski

Other

- Enjoy Your Cells Coloring Book, by Fran Balkwill and Mic Rolph
- Orphan: The Quest to Save Children with Rare Genetic Disorders, by Philip R. Reilly
- Decoding the Language of Genetics, by David Botstein
- Using R at the Bench: Step-by-Step Data Analytics for Biologists, by Martina Bremer and Rebecca W. Doerge
- Aging: The Longevity Dividend, edited by S. Jay Olshansky, George M. Martin, and James L. Kirkland
- Experimental Design for Biologists, Second Edition, by David J. Glass
- Career Options for Biomedical Scientists, edited by Kaaren Janssen and Richard Sever
Audiobook (Audible or iBook Editions)

*Orphan: The Quest to Save Children with Rare Genetic Disorders*,
by Philip R. Reilly

Websites

Cold Spring Harbor Monograph Archive (www.cshmonographs.org)

Services

bioRxiv, the preprint server for biology (www.bioRxiv.org)
BioSupplyNet, scientific supply directory (www.biosupplynet.com)
EXECUTIVE DIRECTOR’S REPORT

Scientists from around the world have exchanged data and ideas at Cold Spring Harbor Laboratory since its first Annual Symposium more than 80 years ago. The Laboratory’s Press extends the opportunity to communicate what’s new and exciting through its growing list of journals, books, manuals, and digital tools. The goal is to provide scientists with information that is appropriate, authoritative, and affordable.

The Press publishes eight journals and has 200 books in print and electronic form and two web services. In 2015, a new journal joined the list. Subtitled “a journal of precision medicine,” Cold Spring Harbor Molecular Case Studies illuminates the traditional medical case report with advanced laboratory investigations such as genome sequencing.

Confidence in this new publication is sustained by the success and reputation of the other Press journals. Genome Research and Genes & Development remain in the topmost ranks of journals in their fields. RNA and Learning & Memory provide value for more specialized research communities. Perspectives and Protocols gain ground and usage as essential library assets. Online, the Press journals had a record download of more than 13.4 million full-text articles.

Interest in print books is falling among scientists—but under the right circumstances, as in 2015, there can still be sufficient demand to make book publishing financially viable. The best sellers were newly released volumes devoted to the intricacies of epigenetics and the computational analysis of genome sequencing, as well as two perennial favorites, At the Bench, an initiation into experimental science, and the classic compendium of lab techniques, Molecular Cloning.

In 2015, the Press built a new distribution platform that permits direct sales of e-books to individuals and research groups. This new approach has proven popular and will be adopted for many future titles. A highlight of the year was the increasing adoption of bioRxiv, the online distribution service for preprints of research papers in the life sciences, founded in 2013. It permits scientists to make their work immediately available to the research community and receive feedback on draft manuscripts before submitting them to journals. The monthly rate of manuscript submission doubled between May and December and represented more than 1500 institutions in 40 countries. Launched with seed funding from the Laboratory, bioRxiv has since received critical support from The Lourie Foundation. Its emergence demonstrates that the Laboratory is continuing to pioneer new ways of advancing science by sharing results and ideas.

Staff

During the year, we said farewell to Delia King following a combined 24 years of service in the Office of the President and CEO and more recently at the Press. We also said farewell to Keri Paterakis, Assistant Operations Manager, after 8 years. We welcomed Laureen Connell back to the Press in a dual role as Senior Editor for Genes & Development and Associate Editor for Molecular Case Studies. Jesse Ulloa, Staff Accountant, and Dorothy Oddo, Administrative Assistant for Genes & Development, joined the Press as well in 2015. A full list of Press staff members as of December 31, 2015, is included elsewhere in this volume.
The mission of the Press is to create publications and services that help scientists succeed while contributing to the Laboratory’s financial support and reputation in scientific education and communication. We can do so only with the engagement of the world’s most accomplished scientists and relationships made possible by respect for the skills and dedication of the Press staff. The Laboratory is fortunate to have them. I thank in particular the individuals with leadership roles in our diverse activities: Assistant Director Richard Sever, journal editors Terri Grodzicker and Hillary Sussman, and departmental directors Jan Argentine, Wayne Manos, Stephen Nussbaum, Marcie Siconolfi, and Linda Sussman. And as always, I am extremely grateful for the cheerfulness, warmth, and efficiency of my Executive Assistant, Mala Shwe Mazzullo.

John Inglis  
Executive Director  
and Publisher
## CONSOLIDATED BALANCE SHEET
December 31, 2015
(with comparative financial information as of December 31, 2014)

<table>
<thead>
<tr>
<th>Assets:</th>
<th>2015</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cash and cash equivalents</td>
<td>$ 54,209,176</td>
<td>56,309,959</td>
</tr>
<tr>
<td>Grants receivable</td>
<td>9,545,355</td>
<td>10,551,528</td>
</tr>
<tr>
<td>Contributions receivable, net</td>
<td>30,100,043</td>
<td>58,786,259</td>
</tr>
<tr>
<td>Investments</td>
<td>449,931,993</td>
<td>442,830,529</td>
</tr>
<tr>
<td>Investment in employee residences</td>
<td>6,161,403</td>
<td>5,159,378</td>
</tr>
<tr>
<td>Restricted use assets</td>
<td>5,412,103</td>
<td>5,127,815</td>
</tr>
<tr>
<td>Other assets</td>
<td>10,270,883</td>
<td>9,983,360</td>
</tr>
<tr>
<td>Land, buildings, and equipment, net</td>
<td>230,619,980</td>
<td>231,650,890</td>
</tr>
<tr>
<td><strong>Total assets</strong></td>
<td><strong>$ 796,250,936</strong></td>
<td><strong>820,399,718</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Liabilities and net assets:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Liabilities:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accounts payable and accrued expenses</td>
<td>$ 10,048,146</td>
<td>12,510,995</td>
</tr>
<tr>
<td>Deferred revenue</td>
<td>8,004,642</td>
<td>5,509,689</td>
</tr>
<tr>
<td>Interest rate swap</td>
<td>34,052,132</td>
<td>33,623,553</td>
</tr>
<tr>
<td>Bonds payable</td>
<td>95,608,887</td>
<td>95,542,618</td>
</tr>
<tr>
<td><strong>Total liabilities</strong></td>
<td><strong>147,713,807</strong></td>
<td><strong>147,186,855</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Net assets:</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrestricted</td>
<td>342,262,835</td>
<td>325,723,978</td>
</tr>
<tr>
<td>Temporarily restricted</td>
<td>192,160,567</td>
<td>236,314,595</td>
</tr>
<tr>
<td>Permanently restricted</td>
<td>114,113,727</td>
<td>111,174,290</td>
</tr>
<tr>
<td><strong>Total net assets</strong></td>
<td><strong>648,537,129</strong></td>
<td><strong>673,212,863</strong></td>
</tr>
</tbody>
</table>

| Total liabilities and net assets             | **$ 796,250,936** | **820,399,718** |

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**FINANCIAL STATEMENTS**

**CONSOLIDATED BALANCE SHEET**

December 31, 2015
(with comparative financial information as of December 31, 2014)

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<td><strong>Total net assets</strong></td>
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<td><strong>673,212,863</strong></td>
</tr>
</tbody>
</table>

| Total liabilities and net assets             | **$ 796,250,936** | **820,399,718** |
CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2015
(with summarized financial information for the year ended December 31, 2014)

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<th>Permanently Restricted</th>
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<th>2014 Total</th>
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<td>Revenue and other support:</td>
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<td>–</td>
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<td>17,887,633</td>
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<td>Rooms and apartments</td>
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<td>Miscellaneous</td>
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<td>Net assets released from</td>
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<td>restrictions</td>
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<td>Total revenue and other</td>
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<td>138,188,198</td>
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<td>Expenses:</td>
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<td>Research</td>
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<td>Educational programs</td>
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<td>DNA Learning Center programs</td>
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<td>1,973,519</td>
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<td>Watson School of Biological</td>
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<td>–</td>
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<td>3,422,312</td>
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<td>General and administrative</td>
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<td>–</td>
<td>17,204,666</td>
<td>16,603,749</td>
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<td>Dining services</td>
<td>5,924,858</td>
<td>–</td>
<td>–</td>
<td>5,924,858</td>
<td>5,704,911</td>
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<tr>
<td>Total expenses</td>
<td>145,050,112</td>
<td>–</td>
<td>–</td>
<td>145,050,112</td>
<td>141,602,706</td>
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<tr>
<td>Excess (deficiency) of revenue and other support over (under) expenses</td>
<td>24,662,127</td>
<td>(32,340,854)</td>
<td>2,939,437</td>
<td>(4,739,290)</td>
<td>(3,414,508)</td>
</tr>
<tr>
<td>Other changes in net assets:</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Investment return excluding</td>
<td>(7,694,691)</td>
<td>(11,813,174)</td>
<td>–</td>
<td>(19,507,865)</td>
<td>(31,486)</td>
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<tr>
<td>amount utilized</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Change in fair value of interest rate swap</td>
<td>(428,579)</td>
<td>–</td>
<td>–</td>
<td>(428,579)</td>
<td>(15,010,072)</td>
</tr>
<tr>
<td>Increase (decrease) in net assets</td>
<td>16,538,857</td>
<td>(44,154,028)</td>
<td>2,939,437</td>
<td>(24,675,734)</td>
<td>(18,456,066)</td>
</tr>
<tr>
<td>Net assets at beginning of year</td>
<td>325,723,978</td>
<td>236,314,595</td>
<td>111,174,290</td>
<td>673,212,863</td>
<td>691,668,929</td>
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<tr>
<td>Net assets at end of year</td>
<td>$ 342,262,835</td>
<td>192,160,567</td>
<td>114,113,727</td>
<td>648,537,129</td>
<td>673,212,863</td>
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# CONSOLIDATED STATEMENT OF CASH FLOWS

## Year ended December 31, 2015

(with comparative financial information for the year ended December 31, 2014)

<table>
<thead>
<tr>
<th></th>
<th>2015</th>
<th>2014</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cash flows from operating activities:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Decrease in net assets</td>
<td>$(24,675,734)</td>
<td>$(18,456,066)</td>
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<tr>
<td><strong>Adjustments to reconcile change in net assets to net cash provided by operating activities:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Change in fair value of interest rate swap</td>
<td>428,579</td>
<td>15,010,072</td>
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<tr>
<td>Depreciation and amortization</td>
<td>13,808,887</td>
<td>13,942,830</td>
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<tr>
<td>Donated equipment</td>
<td>(1,880,032)</td>
<td>–</td>
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<tr>
<td>Amortization of deferred bond costs</td>
<td>66,269</td>
<td>66,269</td>
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<tr>
<td>Net depreciation (appreciation) in fair value of investments</td>
<td>4,238,813</td>
<td>(12,908,048)</td>
</tr>
<tr>
<td>Contributions restricted for long-term investment</td>
<td>(3,057,415)</td>
<td>(5,020,506)</td>
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<tr>
<td><strong>Changes in assets and liabilities:</strong></td>
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<tr>
<td>Grants receivable</td>
<td>1,006,173</td>
<td>(2,045,360)</td>
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<tr>
<td>Contributions receivable, net</td>
<td>25,557,066</td>
<td>48,392,029</td>
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<tr>
<td>Restricted use assets</td>
<td>(284,288)</td>
<td>(623,048)</td>
</tr>
<tr>
<td>Other assets</td>
<td>(287,523)</td>
<td>2,357,289</td>
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<tr>
<td>Accounts payable and accrued expenses, net of financing activities</td>
<td>(1,848,350)</td>
<td>959,182</td>
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<tr>
<td>Deferred revenue</td>
<td>2,494,953</td>
<td>299,798</td>
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<tr>
<td><strong>Net cash provided by operating activities</strong></td>
<td>$15,547,399</td>
<td>$41,974,441</td>
</tr>
</tbody>
</table>

| **Cash flows from investing activities:** |           |           |
| Capital expenditures             | (10,897,945) | (13,605,145) |
| Proceeds from sales and maturities of investments | 44,262,648 | 75,649,786 |
| Purchases of investments         | (55,602,925) | (121,501,780) |
| Net change in investment in employee residences | (1,002,025) | (810,514) |
| **Net cash used in investing activities** | $(23,240,247) | $(60,267,653) |

| **Cash flows from financing activities:** |           |           |
| Contributions restricted for long-term investment | 2,939,438 | 4,001,838 |
| Contributions restricted for investment in capital | 117,977 | 1,018,668 |
| Decrease in contributions receivable | 3,149,150 | 3,209,572 |
| (Decrease) increase in accounts payable relating to capital expenditures | (614,499) | 1,114,499 |
| **Net cash provided by financing activities** | $5,592,066 | 9,344,577 |

| **Net decrease in cash and cash equivalents** | (2,100,783) | (8,948,635) |
| **Cash and cash equivalents at beginning of year** | $56,309,959 | 65,258,594 |
| **Cash and cash equivalents at end of year** | $54,209,176 | 56,309,959 |

| **Supplemental disclosure:** |           |           |
| Interest paid                 | $3,978,881 | 4,013,111 |

## Table Breakdown

- **Cash Flows from Operating Activities**
  - Decrease in net assets: $24,675,734 (2015) vs. $18,456,066 (2014)
  - Adjustments:
    - Change in fair value of interest rate swap: $428,579 (2015) vs. $15,010,072 (2014)
    - Depreciation and amortization: $13,808,887 (2015) vs. $13,942,830 (2014)
    - Donated equipment: ($1,880,032) (2015) vs. $0 (2014)
  - Changes in assets and liabilities:
    - Other assets: ($287,523) (2015) vs. $2,357,289 (2014)
    - Accounts payable and accrued expenses, net of financing activities: ($1,848,350) (2015) vs. $959,182 (2014)
  - Net cash provided by operating activities: $15,547,399 (2015) vs. $41,974,441 (2014)

- **Cash Flows from Investing Activities**

- **Cash Flows from Financing Activities**
  - Decrease in contributions receivable: $3,149,150 (2015) vs. $3,209,572 (2014)
  - Net cash provided by financing activities: $5,592,066 (2015) vs. $9,344,577 (2014)

- **Net Decrease in Cash and Cash Equivalents**
  - ($2,100,783) (2015) vs. ($8,948,635) (2014)

- **Cash and Cash Equivalents at Beginning of Year**

- **Cash and Cash Equivalents at End of Year**

- **Supplemental Disclosure**
FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the Dolan DNA Learning Center receive a substantial portion of 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 2015.

GRANTS January 1–December 31, 2015

COLD SPRING HARBOR LABORATORY

<table>
<thead>
<tr>
<th>Grantor Program/Principal Investigator</th>
<th>Duration of Grant</th>
<th>2015 Funding</th>
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<tr>
<td><strong>FEDERAL GRANTS</strong></td>
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<tr>
<td><strong>NATIONAL INSTITUTES OF HEALTH</strong></td>
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<tr>
<td><em>Equipment Support</em></td>
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<tr>
<td>Dr. Furukawa</td>
<td>04/21/15–03/31/16</td>
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<td>Dr. McCombie</td>
<td>05/01/15–04/30/16</td>
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<td>08/17/11–07/31/16</td>
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<td><em>Cooperative Research Agreement Support</em></td>
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<td>09/21/12–07/31/16</td>
<td>2,261,992.00</td>
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<td>Dr. Kepecs</td>
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<td>Drs. Krasnitz/Wigler</td>
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<td>Dr. Furukawa</td>
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<td>Dr. McCombie</td>
<td>08/16/13–06/30/16</td>
<td>615,516.00</td>
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</table>

1Includes direct and indirect costs
2Cooperative research agreement funding amounts include only CSHL’s portion of the award
*New or competing renewal grants awarded in 2015
<table>
<thead>
<tr>
<th>Grantor</th>
<th>Program/Principal Investigator</th>
<th>Duration of Grant</th>
<th>2015 Funding</th>
</tr>
</thead>
<tbody>
<tr>
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<td>Dr. Mitra</td>
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<td>Dr. Oxen</td>
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<td>Dr. Shea</td>
<td>12/08/15 11/30/20</td>
<td>517,236.00 *</td>
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<td>Dr. Sheltzer</td>
<td>09/18/15 08/31/20</td>
<td>411,000.00 *</td>
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<td>Dr. Siepel</td>
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<td>Dr. Siepel</td>
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<td>Dr. Spector</td>
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<td>Dr. Stenlund</td>
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<td>Dr. Tonks</td>
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<td>Dr. Tonks</td>
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<td>Dr. Trotman</td>
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<td>Drs. Turner/Albeanu</td>
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<td>Dr. Vakoc</td>
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<td>Dr. Van Aelst</td>
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<td>Dr. Zador</td>
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<td>Drs. Zhong/Dubnau</td>
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<td>403,920.00</td>
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**Research Subcontracts**

- NIH/Brandeis University Consortium Agreement: Dr. Mitra 09/26/14 05/31/16 298,565.00
- NIH/Certerra, Inc. Consortium Agreement: Dr. Koulakov 01/01/13 12/31/15 75,500.00
- NIH/Cornell University Consortium Agreement: Dr. Siepel 06/01/14 05/31/16 108,499.00 *
- NIH/Emory University Consortium Agreement: Dr. Huang 04/01/14 02/28/18 47,250.00
- NIH/Johns Hopkins University Consortium Agreement: Dr. Joshua-Tor 03/01/15 02/29/20 36,960.00 *
- NIH/New York University Consortium Agreement: Dr. Koulakov 06/01/14 05/31/19 171,677.00
- NIH/The Research Foundation for the State University of New York—Stony Brook Consortium Agreement: Dr. Wigler 05/01/14 04/30/19 130,599.00
- NIH/Rice University Consortium Agreement: Dr. Spector 03/01/15 07/31/16 4,716.23 *
- NIH/Sloan Kettering Institute for Cancer Research Consortium Agreement: Dr. Sordella 09/12/12 08/31/17 148,565.00
- NIH/Thomas Jefferson University Consortium Agreement: Dr. Enikolopov 03/15/14 03/31/15 979.87
- NIH/University of California—Los Angeles Consortium Agreement: Dr. Wigler 09/26/14 05/31/16 199,880.73
- NIH/University of Minnesota Consortium Agreement: Dr. Tuveson 07/01/12 06/30/16 170,486.00

**Fellowship/Career Development Support**

- Dr. Baker 02/01/15 10/31/17 54,194.00 *

*Includes direct and indirect costs
*New or competing renewal grants awarded in 2015
### Financial Support of the Laboratory

#### Grantor

<table>
<thead>
<tr>
<th>Grantor</th>
<th>Program/Principal Investigator</th>
<th>Duration of Grant</th>
<th>2015 Funding</th>
</tr>
</thead>
<tbody>
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<td>A. Bhagwat</td>
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<td>S. Kelly</td>
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<td>07/16/13 to 07/15/16</td>
<td>$27,120.00</td>
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<tr>
<td>T. Nomakuchi</td>
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<td>02/15/15 to 02/14/17</td>
<td>$27,120.00 *</td>
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<td>O. Odoemene</td>
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<td>01/01/15 to 10/31/16</td>
<td>$43,120.00 *</td>
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<td>Dr. Regan</td>
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<td>12/01/15 to 11/30/18</td>
<td>$54,194.00 *</td>
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<tr>
<td>Dr. Wong</td>
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<td>08/01/15 to 07/31/18</td>
<td>$52,406.00 *</td>
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#### Institutional Training Program Support

<table>
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<th>Duration of Grant</th>
<th>2015 Funding</th>
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<td>Dr. Mills</td>
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#### Course Support

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<td>Advanced Sequencing Technologies and Applications</td>
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<td>Advanced Techniques in Molecular Neuroscience</td>
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<td>Empowering Next-Gen Advanced Biomedical Leadership</td>
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<td>Eukaryotic Gene Expression</td>
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#### Meeting Support

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*Includes direct and indirect costs

*New or competing renewal grants awarded in 2015
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1 Includes direct and indirect costs
2 New or competing renewal grants awarded in 2015
## Financial Support of the Laboratory

**Includes direct and indirect costs**

**New or competing renewal grants awarded in 2015**

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<th>2015 Funding</th>
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### UNITED STATES DEPARTMENT OF AGRICULTURE

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### UNITED STATES DEPARTMENT OF THE ARMY

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### UNITED STATES DEPARTMENT OF ENERGY

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### MISCELLANEOUS SOURCES OF FUNDING

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*Includes direct and indirect costs

*New or competing renewal grants awarded in 2015
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1Includes direct and indirect costs

*New or competing renewal grants awarded in 2015
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|                                              | Dr. Mills                     | 08/01/13 - 07/31/16 | $212,100.00  *
|                                              | Dr. Zador                     | 09/01/15 - 08/31/16 | $300,000.00  *|
|                                              | Dr. Tuveson                   | 12/17/15 - 12/16/16 | $5,000.00    *|
| John Sobolewski in honor of Kathleen Sobolewski | Dr. Zheng                   | 10/01/13 - 09/30/17 | $150,000.00  |
| The Sontag Foundation                        | Dr. Li                       | 04/22/15 - 04/21/16 | $20,000.00   *|
| Vivienne and Josh Spoerri                    | Dr. Li                       | 06/08/15 - 06/07/16 | $393.38      *|
| St. Luke’s School                           | DRS. Hannon/Atwal             | 01/01/14 - 12/31/16 | $396,000.00  |
| Starr Cancer Consortium                      | Dr. Krainer                   | 01/01/15 - 12/31/16 | $166,000.00  *|
|                                              | DRS. Trotman/Hicks            | 01/01/15 - 12/31/16 | $249,000.00  *|
|                                              | Dr. Tuveson                   | 01/01/14 - 12/31/16 | $192,000.00  |

*Includes direct and indirect costs

*New or competing renewal grants awarded in 2015
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**Fellowship Support**

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*Includes direct and indirect costs

*New or competing renewal grants awarded in 2015
### Financial Support of the Laboratory

#### Grantor

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| Manhasset Women's Coalition Against Breast Cancer | Dr. Diermeier                                         | 01/01/15 12/31/15 | $100,000.00 | *
| Maria Nattestad                               | Watson School of Biological Sciences                   | 01/01/15 12/31/15 | 1,000.00    | *
| The Research Foundation for State University of New York | J. Levine                                         | 09/15/15 09/14/16 | 4,200.00    | *
| The Rockefeller University                   | Watson School of Biological Sciences                   | 01/01/15 12/31/15 | 500.00      | *
| Damon Runyon Cancer Research Foundation      | Dr. Chio                                              | 10/01/13 09/30/17 | 52,000.00   | *
| The Simons Foundation                         | Dr. Chen                                              | 03/01/15 02/28/18 | 75,000.00 | *
| Swiss National Science Foundation             | Dr. Musall                                            | 08/01/15 01/31/17 | 70,000.00 | *
| The Swartz Foundation                         | Drs. Albeau/Anselmi                                   | 01/01/15 12/31/15 | 60,000.00 | *
| The Swartz Foundation                         | Drs. Kepecs/Vaughan                                   | 01/01/15 12/31/15 | 60,000.00 | *
| The Swartz Foundation                         | Drs. Zador/Carnevale                                  | 01/01/15 12/31/15 | 60,000.00 | *
| Vasterbottens Lans Landsting                  | Dr. Olund                                             | 01/01/14 12/31/16 | 36,106.50 | *
| New York State Department of Economic Development | Dr. Mills/Post Doctoral Cancer Research Training       | 11/01/12 10/31/17 | 105,699.00 | *
| Lita Annenberg Hazen Foundation               | Watson School of Biological Sciences                   | 05/01/08 04/30/18 | 10,000.00  | *
| William Townsend Porter Foundation            | Undergraduate Research Support                        | 04/01/15 03/31/16 | 13,500.00  | *
| Burroughs Wellcome Fund                       | Imaging Structure and Function in the Nervous System  | 01/01/15 12/31/15 | 10,000.00  | *
| The Leona M. & Harry B. Helmsley Charitable Trust | Course Support                                      | 02/01/15 01/31/18 | 1,100,000.00 | *
| Howard Hughes Medical Institute               | Course Support                                        | 08/01/15 07/31/19 | 600,000.00 | *
| The Lustgarten Foundation                     | Workshop on Pancreatic Cancer                         | 02/01/15 01/31/16 | 10,000.00 | *
| The Nancy Lurie Marks Family Foundation       | Workshop on Autism Spectrum Disorders                 | 07/01/12 06/30/16 | 25,000.00 | *
| Pancreatic Cancer Action Network, Inc.        | Workshop on Pancreatic Cancer                         | 02/01/15 01/31/16 | 10,000.00 | *
| Society for Neuroscience/International Brain Research Organization | Summer Neuroscience Course | 07/01/15 06/30/16 | 12,055.64 | *
| Karen E. Zoller                               | Course Support                                        | 12/14/15 12/13/16 | 5,000.00   | *
| Calico Life Sciences LLC                      | Molecular Genetics of Aging                           | 12/30/15 12/29/16 | 5,000.00    | *
| Carl Zeiss Microscopy, LLC                    | Single Cell Analysis                                  | 08/24/15 08/23/16 | 5,000.00    | *
| Diagenode                                     | Genome Engineering: the CRISPR/Cas Revolution         | 04/01/15 03/31/16 | 1,500.00    | *
| Editas Medicine, Inc.                         | Genome Engineering: the CRISPR/Cas Revolution         | 04/01/15 03/31/16 | 10,000.00   | *
| Fluidigm Corporation                          | Single Cell Analysis                                  | 08/24/15 08/23/16 | 26,000.00   | *
| Gilead Sciences, Inc.                         | Stem Cell Biology                                     | 10/01/15 09/30/16 | 5,000.00    | *
| Iowa State University                         | Retroviruses                                          | 05/01/15 04/30/16 | 12,500.00   | *
| Life Technologies Corporation                | Biology and Genomics of Social Insects               | 05/01/15 04/30/16 | 9,550.00    | *
| National Multiple Sclerosis Society           | Genome Engineering: the CRISPR/Cas Revolution        | 04/01/15 03/31/16 | 6,000.00    | *
| New England Biolabs, Inc.                    | Glia in Health and Disease                            | 09/01/15 08/31/16 | 5,000.00    | *
| Qiagen GmbH                                   | Genome Engineering: the CRISPR/Cas Revolution        | 04/01/15 03/31/16 | 1,000.00    | *
| Qiagen GmbH                                   | Single Cell Analysis                                  | 08/24/15 08/23/16 | 5,000.00    | *

1Includes direct and indirect costs

*New or competing renewal grants awarded in 2015
<table>
<thead>
<tr>
<th>Grantor</th>
<th>Program/Principal Investigator</th>
<th>Duration of Grant</th>
<th>2015 Funding¹</th>
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<td>Mr. Niels Nielsen</td>
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¹Includes direct and indirect costs
²New or competing renewal grants awarded in 2015
# DNA LEARNING CENTER GRANTS

<table>
<thead>
<tr>
<th>Grantor</th>
<th>Program</th>
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<th>2015 Funding†</th>
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<td>National Science Foundation, University of Arizona</td>
<td>iPlant Collaborative: Cyberinfrastructure for the Life Sciences</td>
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<td>Albert Einstein Healthcare Network</td>
<td>Gene Screen App</td>
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<td>Bank of America Charitable Foundation</td>
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<td>Beijing No. 166 High School</td>
<td>Chinese Collaboration Agreement</td>
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<td>Scholarships for Minority and Underserved Students in the Central Islip Union Free School District</td>
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<td>Pinkerton Foundation</td>
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The following schools and school districts each contributed $1,000 or more for participation in the *Curriculum Study* program:

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<th>School/District</th>
<th>Contribution</th>
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<td>East Meadow Union Free School District</td>
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<tr>
<td>East Williston Union Free School District</td>
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<tr>
<td>Elwood Union Free School District</td>
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<tr>
<td>Fordham Preparatory School</td>
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<tr>
<td>Garden City Union Free School District</td>
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<tr>
<td>Half Hollow Schools Central School District</td>
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<td>Harborfields Central School District</td>
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<td>Herricks Union Free School District</td>
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<td>Island Trees Union Free School District</td>
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<td>Jericho Union Free School District</td>
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<td>Levittown Union Free School District</td>
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<td>Locust Valley Central School District</td>
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<td>Long Beach City School District</td>
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<td>Massapequa Union Free School District</td>
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<td>North Shore Central School District</td>
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<td>North Shore Hebrew Academy</td>
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<tr>
<td>Oceanside Union Free School District</td>
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<td>Plainview-Old Bethpage Central School District</td>
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<td>Port Washington Union Free School District</td>
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<td>Ramaz Upper School</td>
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<td>Syosset Central School District</td>
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<td>Yeshiva University High School for Girls</td>
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The following schools and school districts each contributed $1,000 or more for participation in the *Genetics as a Model for Whole Learning* program:

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<tr>
<th>School/District</th>
<th>Contribution</th>
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<td>Hicksville Public Schools</td>
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<td>Hofstra STEP</td>
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<td>Horace Mann School</td>
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<td>Huntington Union Free School District</td>
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<td>Incarnation School, New York City</td>
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<td>Island Park Public Schools</td>
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<td>Laurel Hill School</td>
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<td>Merrick Union Free School District</td>
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<td>Mott Hall II, New York City</td>
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<td>North Bellmore Union Free School District</td>
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<td>Oyster Bay–East Norwich Central School District</td>
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<td>P.S. 144, New York City</td>
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<td>Rockville Centre Union Free School District</td>
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<td>Roslyn Union Free School District</td>
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<td>Saint Dominic Elementary School</td>
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<td>Saint Patrick School, Huntington</td>
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<td>Three Village Central School District</td>
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*Includes direct and indirect costs.*
## BANBURY CENTER GRANTS

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<th>Grantor</th>
<th>Program</th>
<th>Duration of Grant</th>
<th>2015 Funding</th>
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<td><strong>FEDERAL SUPPORT</strong></td>
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<td>NIMH Brain Camp VII</td>
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<td>Alkermes, Inc.</td>
<td>Therapeutic Use of Ketamine for Treating Severe Depression: Risks and Potential</td>
<td>2015</td>
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<td>ALS Association of Greater New York</td>
<td>Therapeutic Developments for ALS: Antisense, Therapy, and Stem Cells</td>
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<td>amfAR</td>
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<td>Gilead Sciences, Inc.</td>
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<td>HeritX</td>
<td>Preventing Inherited BRCA Cancer: A Think Tank for Innovative Strategies, Milestone Objectives, and Research Priorities</td>
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<td>Janssen Research &amp; Development</td>
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<td>Thriving with Schizophrenia</td>
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<td>How Can the Genetics and Neurobiology of Borderline Personality Disorder Contribute to Its Diagnosis and Treatment?</td>
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<td>Mt. Sinai Medical School</td>
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<td>How Can the Genetics and Neurobiology of Borderline Personality Disorder Contribute to Its Diagnosis and Treatment?</td>
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<td>Oliver Grace Cancer Fund</td>
<td>Mitochondria and Cancer</td>
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### BANBURY CENTER GRANTS (Continued)

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<td>OBX Inc.</td>
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<td>Pairnomix</td>
<td>Scientific and Clinical Foundation for Precision Medicine in Epilepsy</td>
<td>2015</td>
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<td>The Soref Family</td>
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<td>2015</td>
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<td>State of Maryland</td>
<td>HIV-1 and How to Kill a Killer: Attempts at Total or Functional Cure of HIV-1</td>
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<td>and The Ron and Sanne Higgins Epilepsy Research Fund</td>
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<td>2015</td>
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CORPORATE SPONSOR PROGRAM
FOR MEETINGS SUPPORT

The Corporate Sponsor Program continues to provide critical funding for the vigorous meetings programs 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.

Raising funds for this program continues to be challenging even as the economy improves. We are thus especially grateful to the companies that continued to support us in 2015:

Agilent Technologies
Bristol-Myers Squibb Company
Genentech Inc.
Life Technologies (part of Thermo Fisher Scientific)
Monsanto Company
New England BioLabs Inc.

We look forward to welcoming the following companies in 2016:

Calico Laboratories
Celgene Corporation
Merck & Co., Inc.
Pfizer, Inc.
Regeneron Pharmaceuticals, Inc.

We are particularly appreciative of Calico Laboratories and Regeneron for their three-year commitment to the Corporate Sponsor Program.

The members of the Program receive special privileges in appreciation of their contributions and 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.
DEVELOPMENT

The year 2015 was memorable for Cold Spring Harbor Laboratory as we marked 125 years of world-leading science research and education. It was a time to reflect on our many accomplishments and discoveries and the significant impact that the Laboratory has had on the world. As we now look toward the future, we hope to not only continue our contribution to the basic understanding of living things but also to integrate this knowledge with clinical efforts for the development of novel therapeutic strategies against human disease and the improvement of human health.

As part of the 125th Anniversary Campaign, the Laboratory’s Cancer Therapeutic Initiative has been a huge step forward as our scientists work to translate their basic research to the clinic where they can have a real impact on the treatment and prevention of disease. The building of a Pre-Clinical Experimental Therapeutics facility (PETx) generously funded by David Koch, New York State, and an anonymous donor, and the soon-to-be renovated Demerec Laboratory will aid in CSHL’s goals. Additionally, the recent alliance with Northwell Health (formerly North Shore-LIJ) will give Laboratory scientists greater access to patient samples.

In 1990, Laboratory Honorary Trustee Alan Seligson and his wife Edith established the Andrew Seligson Memorial Fellowship in memory of their son. These grants provided an opportunity for clinicians who have finished their training to do a research fellowship at the Laboratory and gain important basic research skills. With a renewed focus on the integration of clinical and basic research, Trustee Jeff Kelter and his wife Jenny recently made a significant gift to the Laboratory toward permanently endowing a Clinical Fellows Program. The Fund will provide a perpetual resource to enable clinicians from leading hospitals in the United States and abroad to work with research scientists at the Laboratory on the advancement of new cancer therapeutics and diagnostics.

In June, the Helix Society, a group of our most loyal donors who have included the Laboratory in their estate plans, attended an annual lunch held at the newly opened Nicholls Biondi Hall. Helix Society members heard from Laboratory scientist Dr. Stephen Shea about his neuroscience research and his personal journey fighting a debilitating disease. We happily heard that new members have joined the group, including a former neighbor, the parent of a “Partners for the Future” participant (an educational program for high school students on Long Island), and a former Laboratory scientist.

Among our most significant events each year, the Double Helix Medals raises critical unrestricted funds at the Laboratory. In honor of our special anniversary, this year’s dinner was held at the American Museum of Natural History, where we honored David Botstein, Katie Couric, and Anne Wojcicki. Each of these remarkable people spoke about their unique contributions to improving human health. It was a spectacular event where more than $4,500,000 was raised, pushing our annual fundraising to nearly $7,000,000.

We look forward to 2016 with much optimism and hope for a healthier world. Thank you for all of your continued support.

Charles V. Prizzi, Vice President
DNA Learning Center Corporate Advisory Board

The Corporate Advisory Board (CAB) comprises business leaders from the tristate community and is a vital source of funding and outreach for Cold Spring Harbor Laboratory (CSHL). Board members are the driving force behind CSHL’s annual golf outing at Piping Rock Club, which raises critical unrestricted funding for research and education programs. CAB President Eddie Chernoff chaired the 22nd annual CSHL outing, which raised more than $260,000 and honored Cold Spring Harbor Laboratory Association (CSHLA) former president Trudy Calabrese and her husband, founding CAB member Tom. The CAB was first to view the Ötzi the Iceman exhibit now on display at CSHL’s DNA Learning Center.

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CSHL President Bruce Stillman, CAB and Golf Chair Eddie Chernoff, and Bhuve Singh at the 22nd Annual Golf Tournament held at Piping Rock Club

Former CSHLA President and 2015 honoree Trudy Calabrese with Lynne Stahler, Joyce Gay, and Gina Jacobsen at this year’s golf tournament
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2015 Double Helix honorees Katie Couric (far left), David Botstein (second from right), and Anne Wojcicki (far right) with CSHL President Bruce Stillman

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Chris Pendergast (center) and Frank Verdone (far right) of Ride for Life presented a $300,000 check to Drs. Molly Hammell (second from left) and Josh Dubnau (second from right) to fund research to investigate the genetic causes of ALS. Karen Orzel is holding the check.
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Eddie Chernoff (center) and members of the CAB hear from DNALC Executive Director Dave Micklos (far right) about the new Ötzi exhibit sponsored by LIREG.

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