Cold Spring Harbor Laboratory

2010 ANNUAL REPORT

COLD SPRING HARBOR LABORATORY

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Contents

Governance

The Laboratory is governed by a Board of Trustees of up to 35 members that meets three or four times a year. Authority to act for the Board of Trustees between meetings is vested in the Executive Committee of the Board of Trustees. The Executive Committee is composed of the Officers of the Board and any other members who may be elected to the Executive Committee by the Board of Trustees. Additional standing and ad hoc committees are appointed by the Board of Trustees to provide guidance and advice in specific areas of the Laboratory's operations.

Representation on the Board of Trustees itself is divided between business and community leaders and scientists from major educational and research institutions.

The Laboratory is chartered as an educational and research institution by the Board of Regents of the Education Department of the State of New York. It is authorized to operate a graduate program under the name "Cold Spring Harbor Laboratory, Watson School of Biological Sciences" and thereat to confer the degrees of Doctor of Philosophy (Ph.D.), Master of Science (M.S.), and Doctor of Science (Sc.D.), Honorary.

It is designated as a "public charity" under Section 501(c)(3) of the Internal Revenue Code.

Committees of the Board

George W. Cutting, Jr.
(1932–2010)

George W. Cutting, Jr., known long to all as "Butch," was born on July 13, 1932, the son of George W. Cutting and Mary Converse Cutting of Warrentown, Virginia. A graduate of the Rensselaer and Brooks Schools, he entered Yale College where he graduated in 1955 as a political science major. After his 1958 marriage to Lucy Pulling at the Chapel of the Millbrook School (Dutchess County, New York), which her father Edward Pulling had founded and long directed, they lived in New York City where Butch was a stockbroker and portfolio manager at Fahnestock and Company. In 1958, he and Lucy moved to Oyster Bay Cove, Long Island, occupying a house that they built off Yellow Cote Road estate land earlier owned by her grandfather, the illustrious banker, Russell Leffingwell. Amidst the idyllic splendor of his estate's meadows and fields, Butch and Lucy raised their four children—George, Jr., Lucy, Cynthia, and Susie.

Liz and I first met Butch and Lucy during the first year of our marriage when Ed and Lucy Pulling invited us down from Harvard for a February weekend to meet members of the Long Island Biological Association (LIBA). Ed was then its President, having taken over the reins from Neville Ford, several years after he and his wife moved to Long Island following his retirement as headmaster of Millbrook. Then, he and his wife moved into Redcote, the wooden-shingled main house of the Leffingwell estate. Later, Liz and I began to interact much with the Cuttings at the East Woods School, immediately across Yellow Cote Road from the Leffingwell land. Butch and Lucy also sent their children there to be educated.

From the moment of my arrival in February 1968 as the Lab's new Director, I had the good fortune to be able to devote almost all of my time to direct its science. All of our fund-raising efforts aimed at private sources in effect were handled by Edward Pulling. He had no use for a development office as long as he was available to make known the Laboratory's needs. At a late-1970s luncheon at the Piping Rock Club, Ed introduced us to Oliver and Lorraine Grace, who several years before had moved nearby into a gracious, old waterfront home on Cove Neck. Oliver, long interested in cancer research, joined the Lab's Board of Trustees in 1983, and his major gift made possible the 1986 opening of our Charles Moore–designed *Oliver and Lorraine Grace Auditorium*. By then, Ed Pulling, at age 86, thought the time had come to retire as President, telling me that he had found the perfect person to succeed him—Butch.

Here, Ed made the perfect choice. He knew that Butch, then in his mid 50s, had the time, vision, and energy to attract even more community support for the Lab's ever-growing research and educational programs. Butch, as LIBA's President, also became in 1986 a member of our Board of Trustees, having an essential role in the Lab's Second Century Campaign (1989–1992), serving as cochairman of its steering body and directing the special gifts committee that focused on the mem-

bers of LIBA. By the conclusion of the Second Century Campaign, more than $50 million were raised, allowing construction of the Neuroscience Center to commence in 1992. From the moment he took over the LIBA presidency, he sensed the need to have its name much more directly relate to the Lab. In 1991, LIBA became the Cold Spring Harbor Laboratory Association (CSHLA).

As President, Butch always displayed his unqualified enthusiasm for the Lab, believing that the Lab had to stop being Long Island's best-kept secret. Joyously, he designed the Lab's first tie. He soon correctly sensed that the Association's long-term future would be best served by a succession of qualified individuals serving as its President. So, in 1993, he turned over the presidency to Mary Lindsay. Upon his concurrent retirement from our Board of Trustees in 1993, he was elected an Honorary Trustee, continuing to regularly attend its meetings for many years.

Although no longer CSHLA President, Butch for many years continued to have an indispensable role in our community fund-raising efforts. Particularly important were his convincing his longtime friend, Skip Hargraves, to head our Planned Giving activities and his temporarily running our ever-growing Development Office after Susan Cooper's move, in 1997, to the Trudeau Institute in Saranac Lake, New York. By then, Butch and Lucy were spending even longer intervals in their summer house in Homer, on the Kenai Peninsula to the southwest of Anchorage. Frozen fish always accompanied them home from their summers of much fishing, allowing them to hold each year much appreciated, salmon-dominated parties for their still many close friends at the Lab.

Butch's last years were greatly diminished by failure of his kidneys. Lucy valiantly held any further decline at bay through skillful overnight dialysis procedures that she watched over while Butch was asleep. To give her father more normalcy, their daughter Lucy heroically donated to her father one of her kidneys. Happily, it was immunologically accepted, raising hope that Butch would have many more years of meaningful life. This, however, was not to be, with a serious heart attack all too soon putting him back in New York Hospital. Following unsuccessful surgical intervention, he died on May 13, 2010. Today, his name remains a permanent fixture through our *George W. and Lucy Cutting Lectureship* in the Watson School and our renaming as "Cutting House" the early 19th-century whaling home on Harbor Road that now serves to house students in the Watson School of Biological Sciences.

Butch's enthusiastic love for all things Lab and his warm kindness to all are sorely missed.

James D. Watson
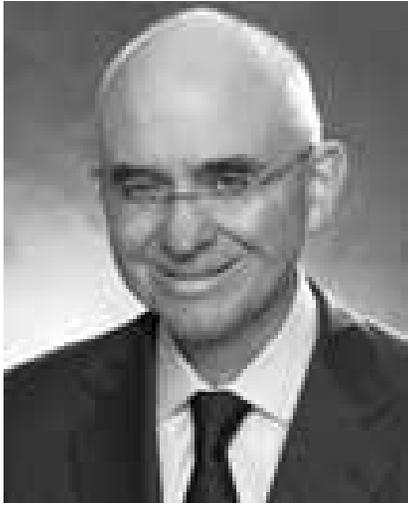
Charles E. Harris III
(1943–2010)

Charlie Harris was remarkable both in how he lived his life and how he died. He and his wife Susan have made invaluable contributions to Cold Spring Harbor Laboratory during the past 20 years that will yield results well into the future.

Charlie was raised in Jacksonville, Florida and attended public school until his parents recognized the need to remove him from the rather dysfunctional public school system. He attended the Hill School in Pottsdown, Pennsylvania for the final 2 years of high school before entering Princeton University. Later, Charlie graduated from Columbia Business School and successfully pursued a career in the securities industry and as an innovative venture capitalist. He founded Harris & Harris Group, a venture capital firm, in 1983 and subsequently brought the firm public. This was an unusual path for a venture fund, but then again, Charlie was unique.

For the last decade of his career at Harris & Harris, Charlie became one of the most informed and enthusiastic supporters of the emerging field of nanotechnology, in which he was recognized as an astute investor. There was something about the merging of an exciting new science and the opportunity to build new companies that attracted Charlie to this field. It enabled him to interact with and advise many of the top nanotechnology scientists in academia. He was always encouraging me to integrate nanotechnology into the research programs at Cold Spring Harbor Laboratory, and he was way ahead of us in this respect.

I know Charlie would have enjoyed this year's President's Council fall event, "Tiny Treatments: The Science of Nanotechnology." He died just the day before the weekend retreat, but he had e-mailed me earlier to say how pleased he was with the agenda. It was Charlie who helped found the President's Council in 1994 as a vehicle to support CSHL Fellows. As a venture capitalist, I believe Charlie looked at the opportunity to support early-career scientists as a smart investment in the future. Investing in the best and the brightest young investigators is a hallmark of our institution, and I view Charlie and Susan's dedication to the CSHL Fellows program as perhaps one of the strongest endorsements of our institutional strategy. The returns on investment from this program are sizable, measurable in terms of scientific advancement and reflected by the grandest of prizes— the Nobel Prize, which in 2009 went to former CSHL Fellow Carol Greider.

Charlie was also an avid thoroughbred horse owner who raced his horses at many prominent tracks, including Belmont and Saratoga. He was often conflicted in time among the alternative careers of family man and father of two, horse owner, businessman, and philanthropist. I remember the Saturday afternoon of one President's Council chaired by Charlie that included a trip to Shelter Island on the east end of Long Island. The drive there required a well-planned stop at a sports bar to watch the Preakness Stakes in Maryland, before we all proceeded to a bird-watching event

with some of the country's top birders. Somehow, Charlie always managed to balance a rather busy schedule and many interests without appearing to be busy.

In 2000, CSHL received endowment funds for The Susan T. and Charles E. Harris Visiting Lectureship, providing students of the Watson School of Biological Sciences the opportunity to interact with the world's leading scientists. Again, I have to believe that with this gift, Charlie and Susan made a strategic investment in the next generation of biomedical research by enhancing the educational experience of some of the brightest doctoral students in the country.

Charlie was elected to the Board of Trustees in 1998 and displayed the same enthusiasm for our science as he did for nanotechnology. He served on many committees including Finance and Investment, Compensation, Development, Audit, and Research. He helped to recruit some of our current trustees and he expanded our network of contacts in both business and academia. We greatly appreciated his sharp intellect and gentle manner.

More recently, we were privileged to see his strengths in a much more personal and profound way. Once diagnosed with cancer in the spring of 2009, Charlie began writing and posting a blog chronicling his treatment, progression, and personal approach and thoughts about dealing with terminal illness. Although he was not trained as a writer, his chronicle became a compelling account of the final chapter of a remarkable life, with deep insights into what it was to live and to face death.

At a memorial service for Charlie on November 3, 2010 in New York, I was touched by the words of so many of Charlie and Susan's friends. The comments of Dr. Andrew W. Lo, Harris & Harris Professor at MIT, brilliantly captured Charlie. "As an academic," said Andrew, "I'm surrounded by colleagues who make it their business to think critically, objectively, and rationally. But I don't think I've ever met a clearer-thinking individual than Charles. He never engaged in the luxury of self-pity, but methodically researched and pursued every avenue of cure that made sense from an overall cost-benefit perspective. Now this may sound cold and calculating, but on the contrary, it was not the cost and benefit to *him* that Charles was weighing but rather the impact on his family and friends."

On June 3, when Charlie decided to stop chemotherapy completely, he began sharing with us all through blog postings his experiences in dealing with his decision and the progress of his cancer. At the urging of his many friends and colleagues, Charlie was persuaded to turn these postings into a book—a project that he worked on up to the end of his life. Entitled "*Incurable: Life After Diagnosis*," the book was published by the Cold Spring Harbor Laboratory Press in 2011. Charlie not only made arrangements to cover the costs of publishing, but stipulated that any profits from the book be dedicated to support research at Cold Spring Harbor Laboratory.

When Charlie was unable to continue writing his blog himself, Susan took over and encouraged us all to meet Charlie's bravery with our own. She and Charlie did not allow us to miss the opportunity to embrace Charlie and celebrate his life while he was still living. Again, I must echo Andrew Lo's beautiful remembrance of Charlie and the gift that he and Susan gave us. We were able to be present in his last months and by allowing us the access to life before death, Susan and Charlie taught us all a lesson on how to live and how to die.

Bruce Stillman

PRESIDENT'S REPORT

In his State of the Union address in 1971, President Richard Nixon called upon Congress "to launch an intensive campaign to find a cure for cancer." Later that year, the National Cancer Act became law, the first salvo in what since has been referred to as "the war on cancer."

After 40 years, where do we stand? This past year, cancers killed more than 550,000 Americans. More than three times that number were newly diagnosed. These figures make clear that a "cure" is nowhere in sight. Yet, four decades ago, it seemed plausible to imagine that we were on the trail of a single killer. Today, we possess the sobering knowledge that our quarry is actually hundreds of different illnesses and that it is unlikely that a single magic bullet will bring cancer's carnage to a halt.

Cancer is so very much more complicated than we understood it to be in 1971. Over four decades, a major national investment in basic biological research—performed at Cold Spring Harbor Laboratory and academic and clinical centers of excellence across the nation and around the world—has yielded increasingly detailed knowledge of cancer at the genetic, cellular, and tissue levels. That knowledge has brought us the first effective targeted therapies for certain cancer subtypes. These point the way to a much more encouraging future.

I would like to recognize in this report a few of the landmark discoveries in which Cold Spring Harbor Laboratory scientists have had important roles, as prelude to describing a new Cancer Therapeutics Initiative. Grounded in such outstanding basic science, I am optimistic that the powerful approach we are taking at the Laboratory will contribute in the coming years to turning many major cancer types into manageable chronic illnesses or even cures.

Forty years is an eternity in biomedical science. It is important to remember that when a patient went to a clinic in 1971, there was very little that an oncologist could determine except for the fact that a cancer was present. Pathology on the tumor could help determine prognosis, but the ability to characterize tumors beyond gross pathology was rather limited. There were plenty of chemotherapies available, but responses to them were essentially hit or miss.

Forty years ago, we knew that the genetics of individual cancers was important. We knew that cancer cells had abnormal chromosomes compared to those of normal cells. But the concept that specific genes caused cancer had not yet been clearly formulated. Our initial focus, beginning in 1968 when Jim Watson became director of Cold Spring Harbor Laboratory and trained his sights on cancer, was on cancer-causing viruses because they carried genes that could promote cancer.

The notion that cancer could have a viral origin dates to the early 20th century and the work of Peyton Rous at The Rockefeller University, who discovered a virus in a type of chicken tumor that could be transferred via injection to baby chicks, which were subsequently observed to develop tumors. In the mid 1970s, J. Michael Bishop and Harold Varmus at UCSF found a gene in healthy chickens called c-*src* that was nearly identical to the cancer-causing gene in Rous sarcoma virus. They concluded that the oncogene in the virus did not represent a true virus gene but instead was a version of the normal cellular gene that the virus had acquired during replication in the host cell and thereafter carried along.

In 1981, Michael Wigler here at Cold Spring Harbor Laboratory was one of three researchers in the United States who independently discovered the first human oncogene, called *RAS*. It belongs to a family of genes critical in signaling networks that regulate cell growth and division. Soon thereafter, CSHL scientist Earl Ruley and MIT's Robert Weinberg began to reveal some of the mechanisms through which oncogenes promote cancer. Their work shed light on the phenomenon of cooperating oncogenes, instances in which the progression of cancer depends on the products of two or more cancer-promoting genes, none of which is sufficient to cause cancer.

This notion dovetailed with the multiple-hit theory of oncogenesis, which led to the idea that cells in our body had to acquire mutations in multiple oncogenes. Following pioneering research by Alfred Knudsen at the Fox Chase Cancer Center, whose studies linked inherited cancer with spontaneous mutations in adult cells and predicted the existence of tumor suppressor genes, Ed Harlow at CSHL demonstrated that oncogenes could inactivate tumor suppressors, thereby providing another view of genetic cooperation to produce tumors. Thus, cancers could result not simply from the actions of cancer-promoting oncogenes—which encoded proteins that accelerated growth within the cell—but also from the simultaneous *absence* of action on the part of genes called tumor suppressors, whose normal function was to *prevent* cellular growth from running amok.

These early studies identified the kinds of malfunctioning or mutated genes that were at work in oncogenesis, and what mechanisms and pathways they undermined to permit uncontrolled cell proliferation and prevention of cell death, both of which were required for tumor progression. In parallel with the genetics of cancer was basic research on cell proliferation control in which many labs at CSHL had a major role and which proved important for understanding cancer. From the mid 1980s to early 1990s, CSHL scientists helped piece together an increasingly comprehensive molecular picture of replication of the genetic material in the cell nucleus and the workings of the cell division cycle that governed how cells proliferate. Defects in the control of cell proliferation are the main drivers of cancer progression, causing increasingly complex mutations in cancer cells that further promote tumor growth, loss of normal controls on cells within a tissue, and eventually metastasis.

In the mid 1970s, CSHL alumni Philip Sharp at MIT, Richard Roberts and Louise Chow at CSHL, and their colleagues made the brilliant discovery of "split genes," Nobel Prize–winning research that enabled us to see how the RNA messages of genes could be spliced together in multiple ways, to generate different proteins from a single gene. As Adrian Krainer has shown in recent years,

this alternate splicing contributes to the emergence of cancer in humans. Most interestingly, Adrian has shown, together with Harvard's Lew Cantley, that the switching by RNA splicing from one form of a gene to another form can endow cells with completely different metabolic outcomes, making cancer cells very different from normal cells. These metabolic changes will likely provide new therapeutic opportunities that exploit basic differences between cancer and normal cells.

With the realization that cancer is fundamentally a genetic disease, it became imperative that we understand the entire human genome. The 1990s marked the beginning of the effort to sequence the human genome and the genomic era in cancer research, and CSHL was among the leaders and innovators. The essence of genomics is captured beautifully in work first performed by Mike Wigler and colleagues around this time. They devised ingenious technical means with which to compare thousands of genes at a time in tumor samples and a patient's corresponding healthy tissue. This immediately led to the discovery of the *PTEN* tumor suppressor gene, mutated in many human cancers. Since 2003, Mike and his collaborators have also called our attention to areas of deletion and amplification across entire genomes, revealing, respectively, a vast array of tumor suppressor genes and oncogenes. This research has introduced a new dimension to the search for the genetic culprits of cancer—phenomena such as gene copy-number variations—not known to exist at this scale before the advent of technologies that study the entire genome.

Amplified and deleted genomic segments in our genome are commonplace. We all have them, and they are often harmless. But when they occur in certain parts of our DNA, the impact can be devastating. Alea Mills of our faculty has provided an excellent example in the context of cancer. Following up on knowledge that a large region of human chromosome 1 was very often deleted in human cancers, Alea was able to determine that the region contained a novel tumor suppressor gene, *CHD5*, that proves to be a master control switch regulating other tumor suppressor genes.

The pace of our insights has grown along with our technological capabilities. It has proven possible to "mine" comparative genomic data obtained from tumor samples to identify, for instance, all overexpressed genes in a particular cancer and then to overexpress the corresponding genes in laboratory mice. It has also been possible to use designer short hairpin RNAs, members of a class of naturally occurring small RNA molecules studied in Greg Hannon's laboratory, to identify many new tumor suppressor genes or to screen for new therapeutic targets in human cancers.

Building upon human genetics research from Mike Wigler, Jim Hicks, and their clinical colleagues Scott Powers and quantitative biologist Alex Krasnitz have identified many genomic regions in human cancer tissue that are either amplified or deleted, enabling insights gleaned from patients to be incorporated into the development of animal models of many cancer types, including liver, colon, prostate, pancreas, and breast cancers, as well as various types of leukemia. In recent years, Scott Lowe and others have made great strides with "mosaic" mouse models, genetic hybrids that use tissue-specific stem cells to introduce quickly into mouse cells the same genetic mutations found in human tumors. These mosaic mice have tumors that mimic the course of human cancers, enabling assessment of why chemotherapy works in some patients and not in others, and validation of whether new therapeutic targets will work on cancers that are resistant to current treatment.

We have learned that the underlying genetics of a tumor determines its response to therapy and can therefore be exploited for both diagnosis and prognosis of tumor subtypes. Carrying this analysis further, Mike Wigler and Jim Hicks developed a method to study genomic heterogeneity *within* a patient's breast tumor, allowing them to identify cellular subpopulations as well as map their spatial organization. This analysis was used to advance our understanding of how a tumor evolves over time, driven by genetic changes that are not visible if the entire tumor is considered to be uniform.

Using powerful RNA-based tools developed at CSHL, we are learning how to identify new targets for cancer therapy and to probe why an existing targeted drug works brilliantly for one patient and fails utterly with another. Previously, both might superficially have appeared to have the same kind of cancer, but now genetic analysis can separate tumor responses into subgroups, even within a particular tumor tissue type. RNA-based technology and cancer genetic techniques are also enabling

CSHL scientists to study closely the perplexing phenomenon of resistance to existing drugs. It is now very clear that new, targeted therapies have to be developed for each genetic subtype of tumor.

Targeted therapies made a huge impact with the development of Gleevec, designed specifically to block an oncoprotein produced by a mutant gene in the so-called Philadelphia chromosome, a misshapen chromosome discovered at the University of Pennsylvania and Fox Chase Cancer Center in 1960 and now understood to be the result of a translocation—a fragment of chromosome 9 fused to a fragment of chromosome 22. Gleevec helps only those patients who have this uncommon mutation, which is the cause of most cases of an acute blood cancer called chronic myelogenous leukemia, or CML.

Similarly, Tarceva is a drug that very specifically blocks the product of a mutant version of a gene called *EGFR* (epidermal growth factor receptor), present in a subset of lung cancer cases. Like Gleevec, Tarceva is not an indiscriminate killer of cells, both cancerous and healthy, like old-line chemotherapies. Rather, it works well in many patients who have a specific EGFR mutation, but it does not help those whose lung cancers have other genetic drivers. However, Tarceva, when effective, typically holds the cancer at bay only for a year or two and then drug resistance emerges. Raffaella Sordella's lab at CSHL recently has found a new mechanism by which responsive lung cancers develop resistance to the drug.

The problem of resistance suggests the difficulty of the task before us and leads me to caution against undue optimism that "a cure" is just around the bend. There are 50-odd major types of human cancers based on tissue type alone, and there are probably six or seven important subtypes within each tissue type (and maybe more), each one of which needs to be treated with what I anticipate will be a cocktail of targeted drugs rather than a single one. Only then will the resistance that cancers naturally develop be avoided. In the not-distant future, therefore, major cancers will be treated in the manner that we now treat HIV infections, with multiple drugs that minimize the development of resistance. For now, therefore, chronic management of cancer is a more realistic prospect than its eradication, and this will be a major advance if the targeted drugs do not cause major side effects, as in the case of Gleevec.

Our Cancer Therapeutics Initiative brings together many of the innovative elements I have discussed here. Beginning, importantly, from human tumor samples—which we obtain through our collaborations with leading clinical centers—we use our state-of-the-art sequencing and genome analysis capabilities to generate tumor profiles. Working with subsets of genes that emerge for genetic analysis of human tumors, RNA interference (RNAi) technologies can rapidly identify the Achilles' heel of the cancers and suggest new therapeutic targets. Validation of these targets in mouse models of human cancer will most likely increase the success rate of drugs that eventually enter into the clinic. We have learned the hard way that there is no substitute for observing the molecular mechanisms of cancer and their response to therapies within the incredibly complex living environment in which actual cancers emerge, grow, and spread.

The net impact of our initiative—which I estimate will cost $100 million over a period of years—will be the ability to systematically discover and rapidly validate new targets for cancer drugs. Such an initiative will require constant interactions with the pharmaceutical industry to bring the validated targets to human clinical studies. This will require seamless interactions among scientists in industry and academia. Academic scientists lack the resources to develop drugs, and given well-validated targets, industry has proven to be very effective at developing drugs that work. The problem is that industry has not been good at discovery of targets with a high probability of clinical success. This is where I expect academia will excel.

While the Cancer Therapeutics Initiative is needed, CSHL will continue vigorously to pursue basic research on small RNAs, genome structure and organization, cellular signaling pathways and networks, and other aspects of fundamental biology, work that will lead us to other new technical capabilities and understanding. It is possible that research performed on our campus will help solve the technical problems that currently prevent us from using RNAi to directly shut down cancer genes

in human patients. Other areas of basic research, notably on the immune system, tumor metabolism, and tumor microenvironment, are likely to be of increasing importance in the years just ahead.

There is one additional element in our fight against cancer that I would like to mention, and it concerns the current state of our clinical trials system. If we and others are successful in identifying novel, very specific drug targets in subtypes of the major cancer killers, it is vitally important that drugs developed against these targets not get bogged down in regulatory delays. A drug recently developed against a comparatively rare genetic mutation in lung cancer gene called *ALK* provides a case in point. A recent early-stage clinical trial of an experimental drug called crizotinib was notably successful in patients with non-small-cell-lung cancer (NSCLC) who harbored the *ALK* mutation, with tumor shrinkage and stabilization in the range of 85%. Strikingly, about three-quarters of the patients remained on the drug after the clinical trial met its endpoint. Under the current system, the FDA will require the drug developer to randomize treatment in a phase III trial, splitting a group of *ALK*-positive patients into two groups, only one of which will receive the drug. The desired endpoint would be to demonstrate a survival advantage, a process that takes years to play out.

Proceeding in this manner I would argue is unethical and costly. In some cases, such as this one, phase III trials could be bypassed. A drug showing overwhelming responses in multicenter, early-stage trials in a cancer type with poor prognosis should promptly be granted temporary approval. It should be placed directly into broad clinical use in appropriate genetically screened patients who wish to be treated with it, including early-stage cancer patients. The drug's developer, meantime, should be required to report the full course of all patients, irrespective of outcome. Hospitals and clinics performing these trials should be protected from patient litigation if the therapies do not work, allowing multicenter trials to proceed unhindered by legal complications. For a period of years, all adverse side effects and outcomes should be reported and the drug's temporary approval rescinded if previously unnoticed safety issues emerge or if the drug proves not to have the desired effect when a larger group of patients have been treated. Short of this, however, I believe humanitarian and cost considerations demand that a new drug found to have overwhelming initial success in a genetically defined subpopulation of patients with otherwise poor prognosis should be made available while further data on efficacy and side effects are being collected.

If we are serious as a society about advancing the state of cancer treatment, we should rethink the clinical trials process, particularly as we use new methods of discovery made possible by decades of remarkable basic scientific and clinical research to find the next generation of targeted therapies. These, if used in combination treatments, promise to make cancer a disease that millions of Americans will be able to live with, while enjoying a decent quality of life. It is not an easy goal, but one that should be among the nation's highest priorities.

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

Research

Research at Cold Spring Harbor Laboratory (CSHL) has a major impact in the areas on which our principal investigators focus: cancer, neuroscience, plant biology, and quantitative biology. It has often been noted that our influence is especially remarkable for an institution of CSHL's comparatively small size. A recent survey by the respected science publisher Thompson Reuters in fact placed CSHL first in a group of 20 "heavy hitters" in molecular biology and genetics, selected from among 42,000 research institutions worldwide. During the first decade of the 21st century, research papers based on work conducted in CSHL laboratories had more impact—as measured by their frequency of citation by peers—than papers originating in any other institution, including the Massachusetts Institute of Technology, the Salk Institute for Biological Studies, Memorial Sloan-Kettering Cancer Center, The Rockefeller University, and Harvard University.

This survey is not the only measure of our worth or that of any institution, but it does suggest the power of the work being performed at CSHL and its relevance, as measured by those who use it—our colleagues at laboratories throughout the nation and across the globe. Together, we are engaged in a vital enterprise, in which we bring all of our intellectual skills and technical ingenuity to bear on fundamental questions of biology and generate knowledge that forms the basis for biomedicine to move forward in its mission to relieve the major causes of human suffering. Below, we summarize just a few of the many fascinating and important findings made by CSHL's dedicated team of investigators during 2010.

Antisense Therapy Reverses Spinal Muscular Atrophy in Mice

Professor Adrian Krainer achieved a milestone this past year in his continuing effort to understand spinal muscular atrophy (SMA), the leading genetic cause of death in infants. SMA is the result of mutations in the survival of motor neuron 1 (*SMN1*) gene. These lead to abnormally low levels of SMN protein in motor nerve cells of the spinal cord and to the degeneration of those cells. Last year, Adrian and colleagues identified a compound that stimulates SMN production by altering RNA splicing. This year, they carried the work an important step further: By introducing chemically modified pieces of RNA called antisense oligonucleotides (ASOs) into the spinal cords of mice, they succeeded in reversing symptoms of Type III SMA. This result exemplifies how superb basic science—in this case, work in the Krainer lab on the cell's splicing ma-

A. Krainer

chinery—can be fertile ground for value-added science, the kind of research activity that adds commercial value to fundamental discoveries. Krainer's team has collaborated with scientists at Isis Pharmaceuticals in designing and synthesizing ASOs, which can be designed to bind to any piece of RNA. The team zeroed in on an ASO that optimally enhanced the inclusion of an exon that in people with SMA is "skipped" by cellular machinery that cuts and pastes bits of RNA "message" together to form a template for protein manufacture. A particularly encouraging aspect of the team's progress this year was learning how to overcome barriers to delivering ASOs directly into the fluid that surrounds the brain and spinal cord. The treatment's therapeutic effect in mice persisted for half a year after it was discontinued, indicating that the ASO is very stable. In addition, the team reported no inflammation or toxicity.

Reversing Alzheimer's-like Memory Loss in Drosophila

Work published this year by Professor Yi Zhong's team demonstrated a means of reversing memory loss in fruit flies caused by brain plaques similar to those implicated in Alzheimer's disease. Modeling a complex human illness such as Alzheimer's is an important goal of basic science, and the fly pro-

vides us with a suitably simple starting point. The fly brain should not be underestimated, for in it we see significant conservation of DNA sequence found in human genes known to affect the structure and function of neural networks. Protein fragments of the β-amyloid molecule associated with Alzheimer's are known to alter many cell-signaling proteins such as phosphoinositol-3 kinase (PI3K), causing a wide range of neuronal dysfunctions. In flies engineered to produce the human β-amyloid protein in their brains, Yi's team set out to better comprehend the molecular basis of memory loss. This yielded a finding that went against received wisdom that attributed a protective role to the kinase. Yi's team instead found that the increased PI3K activity caused a type of neurotransmission that is pathologically enhanced when β amyloid is present in the fly brain. Injection of chemicals that block the kinase's action and separate efforts to turn off the gene that encodes it both had the effect of restoring normal signals in the fly brain. This research also intriguingly suggests that brains affected by Alzheimer's might become insulin resistant because of elevated PI3K activity. Thus, the kinase becomes a potential target for novel therapeutics.

Y. Zhong

A Possible Inflammatory Component in Resistance to a Targeted Lung Cancer Drug

A critical question about cancer concerns the molecular mechanisms involved in resistance to chemotherapy. Particularly vexing is the phenomenon of resistance to the best drugs developed to date, so-called targeted therapies. Assistant Professor Raffaella Sordella's lab this year shed new light on resistance to Tarceva (erlotinib), a targeted therapy approved in 2004 for a subset of patients with non-small-cell lung cancer (NSCLC) and for some patients with pancreatic cancer. Tarceva's molecular target is known—the cell membrane receptor called epidermal growth factor receptor (EGFR)—as are processes that lead to about half of observed cases of resistance. But what about the other 50%? Raffaella and colleagues from Weill Cornell Medical College and the Boltzmann Institute in Vienna discovered 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.

R. Sordella

A Protein Linked to Leukemia "Bookmarks" Highly Active Genes in Dividing Cells

When CSHL Fellow Christopher Vakoc and colleagues demonstrated this year how so-called epigenetic instructions are stably transferred from one generation of cells to the next, they provided a compelling explanation of how a protein called MLL (mixed lineage leukemia) may be involved in triggering leukemia. During cell division, gene activity is normally shut down temporarily. The dividing cell's chromosomes condense and expel most of the proteins that cling to them, which are called epigenetic marks. These marks at other times in the cell cycle help to determine which genes are accessible to the cellular machinery and can be expressed and which genes are inaccessible and cannot be expressed. Unlike most other chromosome-bound epigenetic marks, Chris' team found that the MLL protein stays tethered to the genetic material during cell division. It acts as a "bookmark," preserving a bit of vital gene expression information. But as the cell divides, sometimes the MLL proteins shift to new locations on the chromosome. Interestingly, they seem to attach to genes that are the most active before cell division shuts down all gene activity. This, in turn, can draw

C. Vakoc

other proteins to the same area, with the net effect of jump-starting gene expression. Chris is now studying how MLL mutations might promote the abnormal proliferation of cells in leukemia.

A Potential Way to Reverse Cancer Cell Metabolism and Tumor Growth

Eighty years ago, Nobel laureate Otto Warburg observed the altered metabolic state of cancer cells and tried to connect it, biochemically, with processes that give rise to the rapid proliferation that characterizes cancer. In particular, cancer cells are distinct in the way in which they metabolize glucose. They also produce large quantities of a by-product called lactate. A protein called PK-M2 is a key mediator of glucose metabolism in cancer cells, and this year, Professor Adrian Krainer led a group including researchers at Harvard Medical School and The Broad Institute that discovered three molecular factors contributing to high levels of PK-M2 in cancer cells. PK-M2 is one of two isoforms, or slightly varying versions, of an enzyme called pyruvate kinase. A single gene called *PK-M* gives rise to both, via alternative splicing. Adrian's expertise in splicing helped the team to understand how the benign isoform of the enzyme, PK-M1, is switched off and the dangerous M2 isoform is switched on in cancer cells. By manipulating three known splicing factors, the team was able to halt M2 production and separately to restore production of the benign M1 isoform. This sheds light on the so-called Warburg Effect and points to possible new targets for drugs that might reverse the pathological metabolism of cancer cells.

How Blood Stem Cells Are Maintained in the Bone Marrow Niche

Hematopoietic stem cells (HSCs) have unique abilities that are prized by medical researchers. They can self-renew and develop, or differentiate, into any kind of blood cell, which enables them to replenish the body's entire blood and immune system. Researchers have understood that these qualities are traceable to a distinct locale or niche within the bone marrow that HSCs target, but the identity and function of the niche-forming constituents had not been clearly defined until this past year, when Associate Professor Grigori Enikolopov and colleagues from the medical schools at Harvard, Albert Einstein, and Mount Sinai published a report in the journal *Nature*. HSCs retain their unique features, they observed, in response to signals from another stem cell population, called mesenchymal stem cells (MSCs), that create a supportive bone marrow niche for the HSCs. It was the first demonstration that one type of stem cell could regulate another type of stem cell. In a series of experiments, Grisha and the team discovered that genetic factors essential for HSC maintenance are highly concentrated within neighboring MSCs. They speculate that if we can control the niche, we can also manipulate the HSC population within it. This raises the prospect of developing a drug to target the niche in order to enhance stem cell production. This would be useful in regeneration therapies or could help to prevent the development of certain leukemias and other illnesses related to unregulated stem cell proliferation.

G. Enikolopov

Next-Generation Sequencing Enables Team to Find Cause of Devastating Rare Illness

Professor Gregory Hannon and his talented graduate student Yaniv Erlich—who in 2010 received his Watson School doctorate as well as a prestigious appointment as a Fellow at the Whitehead Institute—were part of an international team that discovered a genetic mutation that causes Joubert syndrome, a rare inherited neurological disease found most often among Ashkenazi Jews. Children whose parents both carry a copy of the mutated gene, and who inherit a copy from each, develop devastating pathologies including malformation of the brain, developmental delay, and muscular and visual impairment. The CSHL contribution to the discovery of the mutation's precise location involved a technological insight. Rather than sequence the entire genome of patients in search of the genetic culprit, which would be time-consuming and very costly, the team could use a

G. Hannon

powerful genome fractionation method devised by Greg's team to sequence only those portions of the genome that encode proteins. This is called the exome, and it consists of less than 2% of the entire human genetic sequence. This was one of the very first instances in which next-generation sequencing was used to find the genetic cause of a rare disease and demonstrates that similar methods can be used to find the causes of other uncommon illnesses that otherwise might not get the attention that their sufferers so desperately need.

Identifying the (Few) Protein Differences between Neanderthals and Modern Humans

A closely related sequencing technology enabled Professor Hannon, postdoctoral researcher Emily Hodges, and others in Greg's lab to play an important part in a story that *Science* called one of 2010's most important. After years of effort, a team led by Svante Pääbo at the Max-Planck Institute in Germany succeeded in piecing together a draft of the full genome of our Neanderthal predecessors. This was notable in part because the bone fragments from which the DNA was sampled were so old—approaching 40,000 years. But it was also remarkable because the fragments were highly corrupted, some containing as little as two tenths of 1% of Neanderthal DNA. One challenge was how to sift such a tiny portion from the corrupted remainder. This was where the Hannon lab's technique called array-capture resequencing proved to be especially useful. They used it to sequence 14,000 genes known to be different in humans and our closest relatives on the tree of life—chimpanzees. Although about three-fourths of the proteins encoded by those genes are different in humans and chimps, Greg's team showed that stunningly few of them differed in humans and Neanderthals. In fact, they found only 88 amino acid differences, correlating with 83 proteins. In that register, at least, we are scarcely different from the "cave men."

A Gene Variant Is Found to Dramatically Boost Tomato Yields and Sweetness

Superb basic science gives rise to perspective-altering discoveries such as the one just described, but it also leads to insights that have immense practical value. An example can be found in the work of Assistant Professor Zachary Lippman, who in collaboration with scientists at Hebrew University in Israel identified a gene that pushes hybrid tomato plants to increase their yield by as much as 60%. Not only is the yield-boosting power of the gene—which works when plants make flowers—active in different species of tomatoes and under a range of environmental conditions, it also can help to boost the yields of many other flowering crops. The team made the discovery while hunting for genes that boost hybrid vigor, a property first noted by Charles Darwin and then rediscovered at CSHL by George Shull a century ago. Hybrid vigor, or heterosis, can be seen when the breeding of two plant varieties gives rise to a new generation with higher yield than either

Z. Lippman

of the parental lines. The key to the spectacularly high yields in Zach's plants was a mutation that leaves only one active copy (instead of the normal two) of the florigen gene, whose function is to instruct plants to cease making leaves and begin making flowers, which in turn produce fruit. Zach tells us, incidentally, that the super-high-yield tomatoes are surprisingly sweet because the florigen mutation also boost plants' sugar production.

An Asexual Path to Limitless Food Plant Yield?

A very different approach to boosting yield in food crops is to bypass sexual reproduction altogether. Indeed, this has been a fantasy of plant breeders for many years. When male and female gametes—sperm and egg—combine randomly to generate a unique seed during sexual reproduction, valuable parental traits that have been selected by breeders are erased. A subset of plants does reproduce asexually, however, through a process called apomixis. The offspring of the common dandelion, for instance, are clones of the parent. In 2010, Professor Rob Martienssen collaborated with scientists in

R. Martiensson

Mexico to try to coax a flowering plant, the mustard plant *Arabidopsis thaliana*, to reproduce via apomixis. Key to the experiment was shutting down the activity of a protein called Argonaute 9. By doing this, the team tricked an *Arabidopsis* ovule into manufacturing multiple gametes, rather than one. These gametes carried the full complement of genetic material for the next generation, then, and not half, as is the case when the plant reproduces sexually. The offspring were, in this sense, clones. Intrigued by the observation that mobile genetic elements, or transposons, seemed to promote sexual reproduction, it seemed logical to Rob and colleagues to find a molecule that could silence transposons—Argonaute 9 is one—and determine whether it inhibited sexual reproduction. They succeeded. The trick now will be to detect whether this approach works in other plants that reproduce sexually and then specifically in the subset on which we rely for food.

A Protein Critical for Activating DNA Replication

My own research group discovered how a protein called DDK, an essential activator of DNA replication, actually triggers DNA replication in cells. DDK (for *D*df4-*de*pendent protein *k*inase) is an enzyme that attaches phosphate molecules to other proteins to modify their activity. We found that it performs this operation, called phosphorylation, on a protein called Mcm4, specifically within a domain that acts as a built-in brake to prevent the DNA double helix from being unwound. The phosphorylation by DDK releases this brake, thus initiating the replication of unwound DNA strands. Because DDK is often deregulated in human cancers, this new understanding of its role in DNA replication may help to shape the development of new cancer therapies. Indeed, anti-DDK drugs have recently been introduced into the clinic. The discovery of this self-inhibitory activity within Mcm4 and the finding that DDK is required to overcome it were a surprise. It leads us to ask, why such complexity? We suspect that it might have evolved in response to the importance of precision and accuracy in DNA replication. This fits with the broad picture that we have assembled over the years of how replication is coordinated and controlled by kinase proteins.

Cold Spring Harbor Laboratory Board of Trustees

The Board of Trustees, which includes up to 35 members, meets in full, executive, and other committee sessions numerous times throughout each year to perform its duties as the governing body of the institution. Many significant developments related to board leadership occurred this year and deserve mention.

On behalf of the board, I would like to thank Eduardo G. Mestre, who served on the board since 2001 and was Chairman from 2004 to 2010. With Eduardo's leadership during the first decade of the 21st century, CSHL achieved unprecedented growth and expansion in infrastructure and programs. Serving on committees ranging from Capital Campaign, Executive, Nominating, Research,

E.G. Mestre, D. Ayres

and Robertson Research Fund, he challenged fellow trustees and the leadership of the Laboratory to think strategically. As a result, we were able to prevail in the face of significant external challenges that threatened support for basic research across the country. I am pleased that he will remain associated with CSHL as an honorary trustee.

On November 6, 2010, the board elected a new Chairman, Jamie C. Nicholls, and new slate of officers: Vice Chairs Robert D. Lindsay, comanaging partner Lindsay Goldberg, and Marilyn Simons, President of The Simons Foundation; Treasurer Leo Guthart, CEO of Topspin Partners; and Secretary Ed Travaglianti, President of TD Bank, Long Island. I look forward to working closely with Jamie, who, as CSHL Treasurer since 2009, has demonstrated her unique ability to translate her business expertise to the nonprofit, academic world.
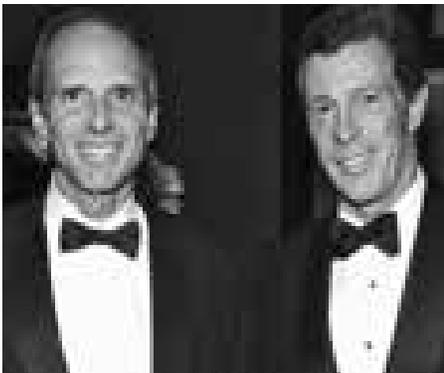
E. Travaglianti, L. Guthart, M.H. Simons, J.C. Nicholls, R.D. Lindsay, B. Stillman

Four new trustees were elected to the CSHL Board of Trustees this year: Tania Baker, Howard Hughes Medical Institute Investigator, E.C. Whitehead Professor, and Codirector of the biology graduate program at Massachusetts Institute of Technology; David Boies, Chairman of the law firm Boies, Schiller and Flexner LLP; Howard Morgan, President of Arca Group Inc. and Director of Idealab; and Dinakar Singh, founding partner of TPG-Axon Capital.

Thank you Lola N. Grace, Vice Chairman from 2004 to 2010, for your enduring commitment to CSHL. Lola retired from the Board this year and was elected an honorary trustee. Lola served on the Board of Trustees since 1995, playing an active part as a member of many committees and providing leadership as an officer since 1998.

We also extend our affection and gratitude for devoted service to retiring trustees Kristina Perkin Davison (2002 to 2010) and Laurence F. Abbott (2004 to 2010).

Two dear friends and former trustees passed away this year. We fondly remember George W. Cutting, Jr. and Charles E. Harris III, who both contributed in unique and generous ways to the growth of CSHL's research and education programs. "Butch" Cutting was instrumental in the formation of the Long Island Biological Association, which was later named the CSHL Association. Butch served on the CSHL Board of Trustees from 1986 to 1993. Charlie served on the Board from 1998 to 2004 and was a founder of the President's Council, created to support the CSHL Fellows program.

CSHL Association

Thank you to the Cold Spring Harbor Laboratory Association (CSHLA) active leadership team of President Tim Broadbent and 25 elected directors, who organized events and letter-writing campaigns to raise $5.6 million of unrestricted funds in support of early-career scientists at CSHL.

This year, more than 140 women gathered at Peacock Point, an exclusive enclave of Long Island's Gold Coast, for the Women's Partnership for Science lecture and luncheon: "Autism: Breaking the Code." The speaker, Alea Mills, Ph.D., has received numerous awards for her work in the field of cancer research and has recently turned her expertise in molecular biology toward understanding the genetic basis of autism. Alea spoke about her recent success in generating a novel mouse model with a chromosomal abnormality that is frequently found in children with autism. These mice, which demonstrate the unique behavioral features of humans with autism, are the subjects of intense

A. Abbott, L. Trafelet, B. Stillman, E. Ainslie, M. Snow, K. Davidson

research in her lab as well as with a team of neurobiologists at CSHL. In its 9th year, the event started by Kristina Perkin Davison has raised more than $500,000 to benefit the research of CSHL's female investigators.

Other friend-raising and fund-raising events initiated by the CSHLA directors included hosting a Regional Junior Chess Tournament and a Major Donor Reception in Old Westbury at the home of Cornelia Guest.

Research Faculty

Awards

Professor and Neuroscience Program Chair Tony Zador was awarded a prestigious $2.17 million Transformative Research grant by the National Institutes of Health. He will use the 5-year research grant to analyze the connectome—the brain's wiring—and determine how its disruption leads to diseases such as autism.

T. Zador

Tony also received one of seven Distinguished Investigator grants from the Paul G. Allen Family Foundation. These—the first of their kind—are part of a program launched by the Foundation to advance important neuroscience and cellular engineering research. Tony, whose grant totals $1.6 million, proposes to develop a highly efficient method for determining the neural wiring diagram for any genetically accessible organism, a crucial requirement for understanding how the brain functions.

Assistant Professor Adam Kepecs was named a John Merck Scholar and received a $300,000 research grant to develop new technologies that would help to reveal the role of the cholinergic nervous system in cognitive tasks involved in learning and attention.

Adam also won recognition in the fall as a finalist for the Eppendorf and Science Prize in Neurobiology. The award recognizes outstanding international neurobiological research by a young, early-career scientist, as described in a 1000-word essay based on research performed within the last 3 years. Dr. Kepecs's essay, entitled "Are you certain? The neural basis for decision confidence," is available online at www.sciencemag.org.

Assistant Professor Raffaella Sordella received the 2010 Damon Runyon–Rachleff Innovation Award to carry out bold, high-risk research to determine the molecular basis of cancer drug resistance and strategies to overcome it, a result that could provide life-changing benefits for a large number of cancer patients.

A. Kepecs

In November, *Genome Technology* asked researchers in the field of systems biology to identify its rising stars. Three of the 24 rising stars on the list were from CSHL. Two are

T. Kelly, L. Goldman, B. Stillman

recent WSBS graduates: Yaniv Erlich, for work in "Fast-Paced Bioinformatics," and Nicholas Navin, for work in "The Evolution of Cancer Tumors." Assistant Professor Michael Schatz was recognized for his work on "Genome Assembly and the Cloud."

I was honored to receive the 2010 Louisa Gross Horwitz Prize from Columbia University with Thomas J. Kelly, M.D., Ph.D., of Memorial Sloan-Kettering Cancer Center, for our work in elucidating mechanisms involved in the process by which DNA—the genetic material contained within the nucleus of nearly all our cells—replicates itself. Tom and I are proud to have contributed to understanding the way cells work in humans and to have shed light not only on the duplication of normal cells, but also on how the process goes awry in cancer.

N. Navin

New Staff

Fritz Henn, Professor, joined CSHL from neighboring research institution Brookhaven National Laboratory (BNL), where he oversaw the biology and medical departments and performed research, often using sophisticated imaging techniques, that has contributed to our knowledge of how the brain functions, particularly in the field of depression. Fritz earned a Ph.D. in physiological chemistry from The Johns Hopkins University in 1967 and an M.D. from the University of Virginia in 1971. He performed his residency in the Department of Psychiatry at Washington University School of Medicine from 1971 to 1974. He began his career at the University of Iowa College of Medicine, and, in 1982, he joined Stony Brook University (SBU), where he became Professor and Chair of the department of psychiatry and behavioral medicine. Following an extensive period in Heidelberg, Germany, Fritz returned to the United States as Deputy Director of BNL before accepting a professorship at CSHL. He has collaborated with Assistant Professor Bo Li.

F. Henn

Anne Churchland, Assistant Professor, joined CSHL after completing her doctorate at University of California, San Francisco, and postdoctoral research in a primate lab at the University of Washington, Seattle. Shifting from primate research to rodent research, she will be studying the circuitry underlying multimodal decision-making, in which animals—rodents—gather evidence from multiple sources, for instance, aural and visual, before making a decision.

Molly Hammell, Assistant Research Professor, comes to CSHL after 5 years as a research associate in genetics and genomics under Victor Ambros at the University of Massachu-

A. Churchland

| M. Hammell | C. Hammell | J. Kinney | M. Schatz |

setts. At CSHL, she is applying prediction algorithms to problems in cancer research. She is also Manager of the CSHL Cancer Center's Bioinformatics Shared Resource.

Chris Hammell, Assistant Professor, did his doctoral work at Dartmouth College and his post-doctoral work in the lab of Victor Ambros at the University of Massachusetts. There, he became interested in the machinery that prepares microRNAs to target specific genes, which they in turn regulate. Using *Caenorhabditis elegans* and forward genetics, he continues to focus on how mutations in this machinery could perturb a given microRNA's gene-regulatory activity so as to give rise to a developmental timing defect and set in motion a chain of events culminating in human illness.

Justin Kinney was named our second Quantitative Biology Fellow. He earned his doctorate in physics from Princeton University and spent the last 2 years in postdoctoral fellowships at Princeton and at CSHL, applying his quantitative skills to biological problems. As a Fellow, he will focus on the question of how sequences of very specific regions in the genome interact with proteins to execute gene expression. He seeks to characterize the sequence–function relationship quantitatively.

Michael Schatz, Assistant Professor, developed methods for large-scale computational analysis of DNA sequencing data at the University of Maryland. He is known for his pioneering use of cloud computing for genomics and for the last several years has helped to run a large National Science Foundation cloud computing project. His research at CSHL will focus on meta-genomics—trying to understand individual genomes within a larger genomic context—and on genome assembly and validation projects.

H. Zheng

Hongwu Zheng, Assistant Professor, earned his Ph.D. in biochemistry at Boston University and completed postdoctoral studies at Harvard Medical School. He focuses on glioblastoma, a brain cancer with a poor prognosis. He uses mice to recapitulate genetic and epigenetic aspects of the cancer and approaches the problem from a developmental perspective. Hongwu is exploring ways to resolve differentiation in cells as a method of halting tumor progression.

Promotions

Congratulations to Dinu Albeanu, who was appointed Assistant Professor. Nicholas Navin was promoted to the position of Research Investigator in the laboratory of Michael Wigler. Dan Levy was promoted to the position of Senior Computer Scientist.

Departures

CSHL is proud of our long history as an incubator for early-career researchers who go on to successful careers all over the world. In 2010, Matthew Vaughn became a Research Associate at Texas Advanced Computing Center in Austin. Sheldon McKay took on the job of Scientific Lead, Engagement Team, iPlant Collaborative at the University of Arizona, Tucson. Professor Michael Zhang moved to Dallas to become Director of the Center for Systems Biology, department of molecular and cell biology, University of Texas, Dallas.

Education Programs

Watson School of Biological Sciences

In the National Research Council (NRC)'s latest assessment of 5000 doctoral programs across 62 fields at 212 universities nationwide, the Watson School of Biological Sciences (WSBS) was ranked between third and 17th across 20 cumulative categories. In the category of citations per publication, CSHL ranked first. The NRC assessment is performed over the period of 10 years, and so this is the first opportunity that the WSBS program has had to be included in this national evaluation.

Ten WSBS students, all of whom matriculated between 2004 and 2006, received their Ph.D.s at the 2010 WSBS Commencement Convocation in April. 2010 graduate Yaniv Erlich won the Fred Hutchinson Cancer Research Center's Harold M. Weintraub Graduate Student Award for outstanding achievement during graduate studies.

National Research Council assessment publication

Honorary degrees were conferred upon Carla Jo Shatz, Ph.D. and Thomas R. Cech, Ph.D. Dr. Cech is a Nobel laureate and pioneer in the study of RNA enzymes and telomerase and was recently President of the Howard Hughes Medical Institute. Dr. Shatz, whose research has helped to establish some of the basic principles of early brain development, is Professor of biology and neurobiology and Director of the Bio-X program at Stanford University School of Medicine. She was also an instructor in our neuroscience advanced courses program.

The 2010 Gavin Borden Visiting Fellow Lecture was presented on March 15 by Dr. Gerald F. Joyce, Dean of the Faculty, Professor, Departments of Chemistry and Molecular Biology, and Investigator, The Skaggs Institute for Chemical Biology, The Scripps Research Institute. The title of the 16th annual CSHL Gavin Borden lecture was "The Origin of Life in the Laboratory."

Meetings and Courses

On April 6, CSHL celebrated the opening of Cold Spring Harbor Asia Conferences in Suzhou, China, a meetings program that aims to be the premier hub for scientists throughout Asia who are exploring the frontiers of molecular biology, biomedicine, and biotechnology. The program kicked off with the first James Watson Cancer Symposium, organized by leading scientists representing important current areas of research: Dr. Xiaodong Wang, a Howard Hughes Medical Institute Investigator affiliated with the University of Texas Southwestern Medical Center and the National Institute of Biological Sciences, Beijing; Dr. Scott W. Lowe of HHMI and CSHL; Dr. Yusuke Nakamura of the University of Tokyo; Dr. Tak Mak of the University of Toronto; and Dr. Karen Vousden

B. Stillman, C.J. Shatz

B. Stillman, T.R. Cech

CSH Asia logo

of the Beatson Institute for Cancer Research in the United Kingdom. This 6-day meeting was followed by the first Francis Crick Neuroscience Symposium, which was similarly organized by leaders in the field: Dr. Z. Josh Huang of CSHL; Dr. Mu-ming Poo of the CAS Institute of Neuroscience, Shanghai and the University of California, Berkeley; Dr. Linda Richards of the University of Queensland, Australia; Dr. Joshua Sanes of Harvard University; and Dr. Keiji Tanaka of the Laboratory for Cognitive Brain Mapping, Riken, Japan. In all, the new CSHL Asia program, which operates from a 600,000-square-foot facility—the Suzhou Dushu Lake Conference Center—hosted 10 meetings and more than 2000 scientists from around the world, but primarily from Pacific Rim countries.

Suzhou is only 60 miles west of a "megacity" even larger than New York—the economic powerhouse of Shanghai, population 20 million. Importantly, the new conference center is less than an hour by high-speed rail from Shanghai and, served by two regional airports, is only a 2- to 3-hour plane ride from Japan, South Korea, Taiwan, and Hong Kong. Singapore and Sydney, Australia, are, respectively, 5 and 10 hours distant by air.

This year marked the 75th anniversary of the Cold Spring Harbor Laboratory Symposia on Quantitative Biology. The 2010 Symposium, with close to 70 talks and attendance of more than 400 scientists, was organized by Terri Grodzicker, David Spector, David Stewart, and me. It focused on the topic of Nuclear Organization and Function. To celebrate the history of the symposia, Jan Witkowski, Jim Watson, and I organized a special 1-day event chaired by Robert Tjian, CSHL alumnus and President, Howard Hughes Medical Institute, called "Biology, Society, and the Future." More than 225 guests attended the lectures presented by world experts including Charles Sawyers, Memorial Sloan-Kettering Cancer Center; Spencer Wells, National Geographic Society; Henry Louis Gates, Harvard University; Mark Bear, Massachusetts Institute of Technology; Story Landis, National Institute of Neurological Disorders & Stroke; Peter Neufeld, The Innocence Project; Craig Venter, J. Craig Venter Institute; and Richard Roberts, New England BioLabs, Inc.

The Symposium on Quantitative Biology has become the cornerstone for our annual program of Meetings and Courses, which in 2010 posted record attendance. A total of 7500 researchers from around the globe attended meetings, and more than 1300 attended training courses on our Long Island campuses. A new experimental laboratory teaching suite funded by the Howard Hughes Medical Institute was opened in our Hillside Laboratories complex.

Opening ceremony, CSH Asia Conferences

CSH Asia poster session

Symposium attendees

Dolan DNA Learning Center

In its second year of operation, the Harlem DNA Lab made significant strides in reaching underserved students and teachers in New York City schools. The statistics speak for themselves: 75% of the 6400 precollege students that attended field trips to the Harlem DNA Lab were African American or Latino; 75% of students attending the Harlem DNA Lab came from Title 1 schools, where 40% or more of students are considered low income.

CSHL's DNA Learning Center led by David Micklos also won acclaim for the success of its iPhone app, "The 3D Brain." With 50,000 downloads, it reached no. 7 among education apps for the iPhone and no. 1 of educational iPad apps.

Cold Spring Harbor Laboratory Press

Since 1933, the CSHL Press has continuously evolved and adapted its publications to best serve the contemporary needs of the scientific community. This year, John Inglis' team launched a new initiative, *CSH Perspectives in Biology*, a monthly online publication comprising reviews spanning the complete spectrum of the molecular life sciences. This new venture is intended to provide the life sciences community with authoritative analyses of progress in emerging areas of molecular, cell, and developmental biology, genetics, evolutionary biology, neuroscience, cancer biology, and molecular pathology. The contributions are written by leading researchers in each field and commissioned by a board of eminent academic editors. Subject Collections gradually accumulate articles as new issues of the journal are published and, when complete, each represents a comprehensive survey of the field that it covers.
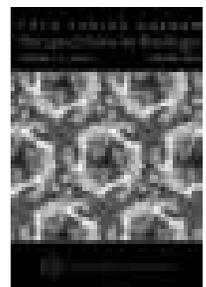
CSH Perspectives in Biology

Development

More than 60 CSHL supporters participated in the Fall President's Council retreat, the 16th year of this event. Participants donated more than $25,000 to support early-career scientists in pursuit of the most promising and innovative research projects. The 2-day retreat immerses these generous philanthropists in the hottest topics in science. This year's event, organized by Diane Fagiola, tackled "The Science of Nanomedicine," with a keynote on the applications of nanotechnology in medicine by Bob Langer, David H. Koch Professor at the Massachusetts Institute of Technology. Other experts included Harvard Professor George Whitesides, University of North Carolina chemistry department

President's Council retreat

head Joseph DiSimone, and Dr. William Sherman of Brookhaven National Laboratory's Center for Functional Nanomaterials.

For the fifth year, 400 guests gathered in early November at the Mandarin Oriental Hotel in Manhattan to honor recipients of the Double Helix Medal—extraordinary individuals who have benefited human health through game-changing biomedical research or by raising awareness and funds for such endeavors. Geneticist Mary-Claire King was honored for outstanding contributions toward understanding the genetics of breast cancer and mental illness. Evelyn H. Lauder received the Medal for her leadership as the Founder and Chairman of The Breast Cancer Research Foundation, which has raised more than $300,000 million to support breast cancer research worldwide. Nobel laureate in Economic Sciences John F. Nash was awarded the Medal for having brought worldwide awareness to and appreciation for people suffering with schizophrenia. The gala event, produced by Charlie Prizzi's team in the development department, raised more than $3 million. A special feature of this event was a musical performance by composer Carter Burwell, whose biography includes a 2-year stint as Chief Computer Scientist at CSHL.

J. Nash, M.-C.King, E. Lauder

CSHL received Charity Navigator's coveted four-star rating for sound fiscal practices, placing CSHL among the most fiscally responsible of more than 1.5 million philanthropic organizations that currently exist in America. This is the ninth consecutive year that CSHL has achieved this top ranking.

Charity Navigator logo

Infrastructure Projects

In June, members of the CSHL community celebrated the completion of an expansion and renovation of the Carnegie building, which dates to the institution's infancy in 1905. In addition to a new state-of-the-art climate-controlled vault for storage of precious archival collections that trace the history of molecular biology and genetics, the updated building now also boasts an annex, named for CSHL alumnus and benefactor Waclaw Syzbalski, Ph.D. Joining us at the opening ceremony was Nobelist Sydney Brenner, who generously donated his archives to CSHL this year.

In November, Art Brings and the facilities department began a project to replace a structure that was originally constructed in 1906 as a green house and potting complex. This structure, which was ultimately named the Hershey Laboratory, after Nobel Prize–winning CSHL scientist Alfred Hershey, was renovated in 1979 to provide offices and to support research activities. The new building currently under construction will be ~18,000 square feet and will house a teaching lab, a seminar room, and a computer classroom for the Laboratory's Meetings and Courses program. It will also be home to the CSHL Cancer Center Shared Resources Flow Cytometry and Microscopy facilities, which have been temporarily moved to the Hillside Laboratories. The Howard Hughes Medical Institute provided $9 million for this project.

CSHL's Sammis Hall, designed by the noted postmodernist architect Charles Moore, was featured in the Heckscher Museum's exhibit, "ARCADIA/SUBURBIA: Architecture on Long Island, 1930–2010." This dormitory residence on the grounds of the Banbury Conference Center was a stop on the house tour associated with the exhibit.

Community Outreach

In celebration of National Lab Day, Professor Gregory Hannon opened the doors of his three-story laboratory building named for Nobelist Barbara McClintock to more than 150 fifth and ninth graders from Long Island and Manhattan schools. With help from his graduate students and postdocs, the visiting children learned to use microscopes to identify lung cancer cells and fruit fly neurons, among other lessons. Greg also spoke about his research endeavors at the February meeting of

Renovated Carnegie building

Hershey Laboratory site rendering

CSHL Partners for the Future

the Secret Science Club in Brooklyn, a monthly gathering of ~400 science enthusiasts primarily from New York City.

CSHL's Partners for the Future program continues to recruit the brightest of Long Island's budding scientists. This year, seven seniors from high schools across Long Island were accepted into the program, giving them daily access to a CSHL mentor and a laboratory in which to conduct a research project of their own. The students devote a large part of their year to working in a laboratory and learning what it is like to be a researcher. At the end of the school year, the students present their findings in a scientific seminar in Grace Auditorium. This year, three students working on cancer-related research projects took an additional step, taking the time to translate their scientific presentations for the public and the media. We offer thanks to the American Cancer Society for partnering with us to publicize this as part of its local Relay For Life events.
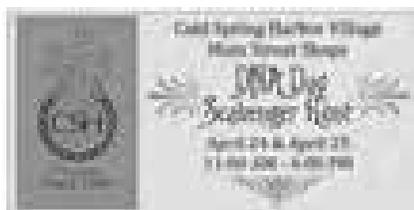
DNA Day Scavenger Hunt poster

Spearheaded by Amanda McBrien, Assistant Director of Instruction at the DNALC, CSHL cohosted a National DNA Day scavenger hunt with local museums and merchants in Cold Spring Harbor village. More than 200 area residents participated in the weekend event celebrating the storied history of the village. April 25 is congressionally designated as National DNA Day, commemorating the completion of the Human Genome Project in April 2003 and the discovery of the double helix structure of DNA in 1953. Each year, the National Human Genome Research Institute encourages national participation in the creation of opportunities for students, teachers, and families to explore the latest developments in genomic research.

The Harlem DNA Lab Instructor Ileana Rios and a banana DNA extraction experiment have become a regular attraction at the World Science Festival Street Fair in Manhattan. CSHL is pleased to have been able to participate once again in this event, which engages many thousands of New York City residents in the celebration of science for a week each June.

Thanks to our students and postdocs who make our CSHL tour program such a success. This year, our tour guides hosted 500-plus visitors. The 2-hour walking tours showcase the history of our science, research, and edu-

Community tours logo

Saturday DNA logo

cation programs. Participants also experience the beauty of the campus' architecture and landscapes.

The CSHL DNA Learning Center's public programs continue to be a hit in the community. During the school year, the DNALC opened its doors on Saturdays for "Saturday DNA," geared to middle and high school students and their parents. During the summer months, more than 900 students in grades 5 through 12 attended week-long camp sessions, on topics ranging from "Fun with DNA" and "World of Enzymes" to "Forensic Detectives" and "Silencing Genomes."

We are thankful for the partnerships that we have with local organizations with whom we interact in many different ways throughout the year. This year, Assistant Professor Hiro Furukawa lectured at a meeting of the Alzheimer's Association of the Long Island Board. We hosted a lecture and tour for the Leukemia & Lymphoma Society board of directors, at which Chris Vakoc spoke. Diane Esposito, Research Investigator in Mike Wigler's laboratory, was an invited speaker on breast cancer research for three different occasions, which were sponsored by the Suffolk County Women's Bar Association, the Women's Center of St. Francis Hospital, and the Adelphi N.Y. Statewide Breast Cancer Program. CSHL representatives cheered on participants in the LI2Day Walk and judged the organization's annual scholarship program. We were also well represented at a local Swim Across America event. We thank these and many other partners who support CSHL through grants and donations.

For details on grants and philanthropic donations received this year, please refer to separate sections of this report. In addition, please visit our newly designed public website, www.cshl.edu for all of the latest news and information about the institution, including electronic access to annual reports, the *Harbor Transcript* magazine, links to stories about us in the media, and postings about research results and education program achievements.

Public Lectures at Cold Spring Harbor Laboratory

March 21—Roger Reeves, Ph.D.: "Theory to Therapeutics in Mice and Men with Down Syndrome," cosponsored by CSHL, the Association for Children with Down Syndrome, the Down Syndrome Connection of Long Island, and the National Down Syndrome Society.

April 26—Thomas R. Cech, Ph.D., Distinguished Professor, University of Colorado-Boulder; Investigator, Howard Hughes Medical Institute; winner, Nobel Prize in Chemistry, 1989: "Learning to Teach, Teaching to Learn."

June 8—Alea A. Mills, Ph.D., CSHL Associate Professor, with **Nikita Anand**, Cold Spring Harbor High School, **Norah Liang**, Patchogue-Medford High School, **Joshua Weiss**, Smithtown High School East: "Next-Generation Cancer Research: Long Island Teens on the Front Line of Discovery."

June 29—Sydney C. Gary, Ph.D., CSHL Director, research operations; **Adam Kepecs, Ph.D.,** CSHL Assistant Professor; **Stephen Dewey, Ph.D.,** The Feinstein Institute of Medical Research; **Edward Nunes, M.D.,** Professor of clinical psychiatry, Columbia University: "Addiction: How the Brain Measures Reward and Response," cosponsored by CSHL, the North Shore–Long Island Jewish Health System, and St. Johnland Nursing Center.

A. Mills

September 26—Steven Allen, M.D., Associate Chief of Hematology, North Shore-LIJ; **Johannes Zuber, M.D.,** CSHL Clinical Research Fellow; **Christopher Vakoc, M.D., Ph.D.,** CSHL Fellow: "Hitting Leukemia Where It Hurts: Researchers and Physicians Unite to Knock Out Cancer," cosponsored by CSHL, The Don Monti Memorial Research Foundation, and the North Shore–Long Island Jewish Health System.

S. Allen, C. Vakoc, J. Zuber

November 14—Nancy Berlinger, Deputy Director and Research Scholar at The Hastings Center: "Ethics of Hope in End-of-Life Care," The Lorraine Grace Lectureship on Societal Issues of Biomedical Research (cancelled due to a sudden unavoidable conflict).

As the Internet continues to evolve, it offers more opportunities for our researchers to speak to the public online. A web magazine, BigThink.com, this year featured interviews of four of CSHL's finest. Interviews are accessible on demand if you visit www.bigthink.com and search for our "experts": James D. Watson, Michael Wigler, Tony Zador, and Adam Kepecs.

Public Concerts at Cold Spring Harbor Laboratory

February 16:	Peter Orth, pianist	**September 3:**	Di Wu, pianist
March 19:	Ran Dank, pianist	**September 24:**	Hahn-Bin, violinist
April 24:	Carducci String Quartet	**October 8:**	Diane Walsh, pianist
May 7:	Soo Bae, cellist	**October 29:**	Aaron Goldberg Trio
May 21:	Einav Yarden and Sergey Ostrovsky, pianist and violinist		

Looking Forward

As we began 2010, our efforts in strategic planning prepared us for a challenging year. We accomplished so much in the face of many external challenges, and this was possible because of the dedication of our more than 1000 employees to excellence in research, education programs, and operations. No doubt, we will continue to face direct challenges related to the recovering economy and leadership changes in federal and state governments, but I am convinced that our continued commitment to excellence will help us to navigate and ultimately prevail. Thanks to all of you who work for and with CSHL.

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

CHIEF OPERATING OFFICER'S REPORT

This was a year of steady progress for the Laboratory. Although there are substantial challenges on the horizon, we take some comfort from the continued recovery in the financial markets since the 2008 debacle, and the resumption of growth, albeit painfully slow, of the U.S. economy.

What keeps Cold Spring Harbor Laboratory's culture so dynamic and invigorating is a sense of constant renewal. As we have absorbed and populated the new Hillside Campus buildings, 11 dynamic new faculty members with diverse research concentrations have joined our community during the last 18 months. Add to this a new class of Ph.D. students at the Watson School, our usual complement of 8000 Meeting and Course attendees, and new scientific publications from the Laboratory Press, and we end up with a unique brew of intellectual capital. Our campus landscape continued to evolve as well, with the groundbreaking construction of the new Hershey Building, made possible by a generous grant from the Howard Hughes Medical Institute.

W. Dillaway Ayres Jr.

It is gratifying to see the asset value of the Laboratory's endowment fund approaching its pre-2008 high watermark. This growth is made possible by a relatively conservative annual spending policy, new donations, and capital appreciation. The investment return for the 2010 calendar year was a positive 10.25% bringing the total value of the fund up to $283 million at year end—this following 20% annual appreciation in 2009. During the last two years, the Investment Committee of the Board of Trustees has sought to reduce the level of risk and volatility in the portfolio by increasing exposure to conservative and proven hedged equity, absolute return, and international equity managers. Although this might cause performance to be slightly below equity indices in bull markets, it should serve the portfolio well over time by providing a prudent balance of capital preservation and growth.

For the fiscal year ending 12/31/10, the Laboratory operating budget reached approximately $147 million—an increase of 14% over the prior year. The substantial revenue growth was largely reflective of compelling research resulting in high success rates with both private and public grant applications. Although this is excellent news, there are caveats of which we must be mindful. Of the federal grant awards received by our investigators, 9% came from funding made available through the American Recovery and Reinvestment Act. These "stimulus" funds, although welcome, are temporary in nature and will not continue beyond 2 years. Of greatest concern is the uncertainty about the future of federal funding for research as the U.S. government struggles with massive deficits and partisan political gridlock. We did achieve our goal of achieving cash-neutral operating results in 2010 after netting depreciation expense against expenditures for capital improvements. However, this was partially achieved by drawing down on the fund for "research start-up" that was prudently raised as part of the $200 million Hillside Campus campaign completed in 2008. This fund is scheduled to be fully spent by 2016. As always, we maintain a lean administration and devote great attention to diligently managing expenses.

The challenges ahead are daunting. Doing cutting-edge research is expensive. Recruiting and retaining the best and brightest investigators while investing in state-of-the-art laboratory equipment and computing infrastructure demands tremendous resources. It is highly likely that the Laboratory will be forced to maintain its level of excellence with less funding from the federal government. We are fortunate to have such a substantial level of private support, but we will need to increasingly rely upon it in the future.

Most importantly, we are inspired by the Laboratory's mission and the work going on around us. And we are ever grateful to our supporters and our loyal staff.

Dill Ayers
Chief Operating Officer

Staff

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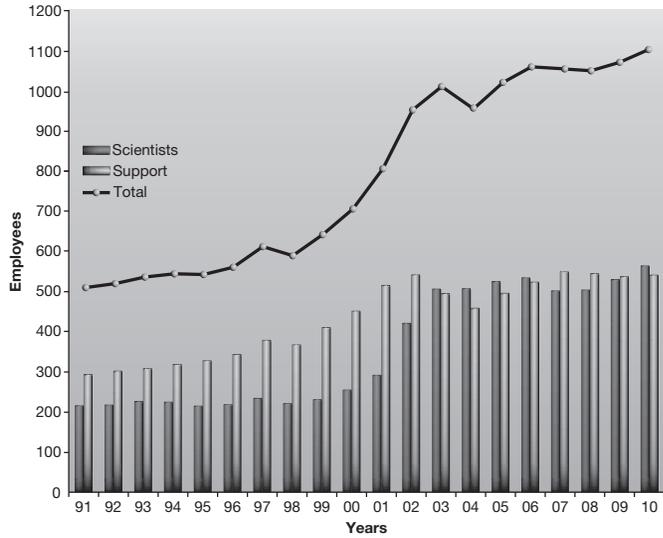
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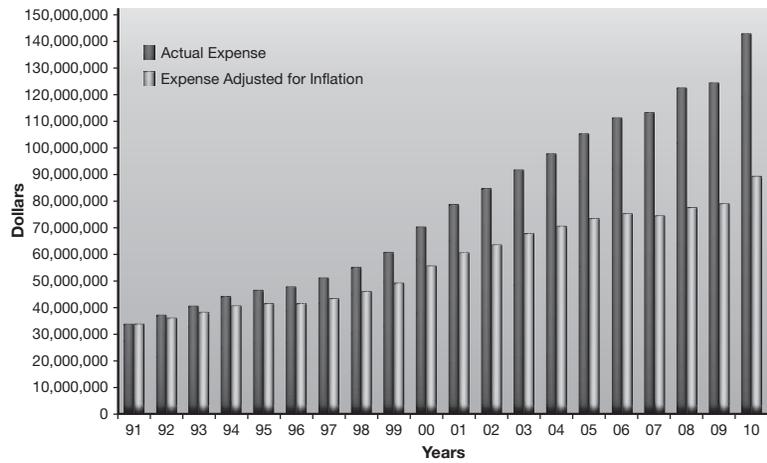
Meetings and Courses Participants
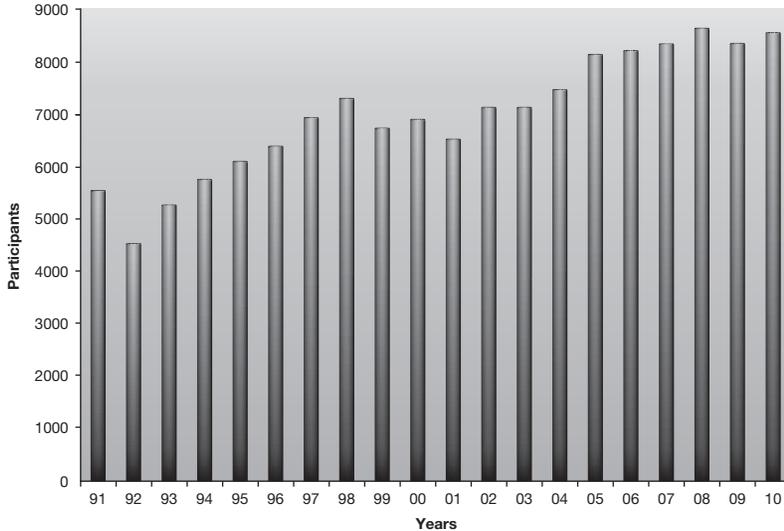
Long-Term Service

Front row (*left to right*) Alyson Kass-Eisler, Linda Rodgers, Maureen Davies, Barbara Matusik, Madeline Wisnewski, Lisa Manche, Barbara Purcell, Leemor-Joshua Tor; *top row* (*left to right*) Edward Campodonico, Bruce Stillman, William Bishop, Barry Burbach, James Watson, Susan Schaefer, Ronald Romani, Giuditta Carino, Andrea Stephenson, Benjamin Veneable, Mona Spector, Bruce Fahlbusch, Nora Ruth, David Spector, Joshua Dubnau

The following employees celebrated milestone anniversaries in 2010:

40 Years	Madeline Wisnewski
30 Years	Bruce Fahlbusch, Linda Rodgers, Andrea Stephenson
25 Years	Frank Carberry, Lisa Manche, David Spector
20 Years	Edward Campodonico, Clare Clark, Beicong Ma, George Newell, Natalia Peunova, Barbara Purcell, Ronald Romani, Yew Teo, Nicholas Tonks
15 Years	William Bishop, Barry Burbach, Giuditta Carino, Kenneth Chang, Jodi Coblentz, Maureen Davies, Joshua Dubnau, Leemor Joshua-Tor, Alyson Kass-Eisler, Scott Lowe, Barbara Matusik, Mary Muno, Nora Ruth, Susan Schaefer, Mona Spector, Benjamin Veneable, Claudia Zago

RESEARCH

See previous page for photos of the following scientific staff:

Row 1: W. Li (Mills lab); R. Solomon (McCombie lab); A. Tolpygo (Mitra lab);
S. Weissmueller (Lowe lab)

Row 2: S. Schuck (Stenlund lab); P. Thekkat, A. Rosebrock, F. Karginov, J. Preall (Hannon lab); K. Creasy (Martienssen lab); C. Johns (Gingeras lab)

Row 3: M. Vigliotti (Lucito lab); A. Mofunanya (Powers lab); Z. Glass (Enikolopov lab); N. Simorowski (Furukawa lab)

Row 4: S. Das (Krainer lab); B. Boivin, X. Zhang (Tonks lab); K. Jiang (Lippman lab); J. Milazzo (Lazebnik lab)

Row 5: E. Plavskin (Timmermans lab); M. Rossman (Stillman lab); T. Schalch (Joshua-Tor lab); M. Mahdavi (Dubnau lab)

CANCER: GENE REGULATION AND CELL PROLIFERATION

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

Through forward genetic approaches in *C. elegans*, Christopher Hammell and colleagues have identified a variety of gene products that modulate the efficacy of and/or regulate the activity of core microRNA (miRNA) machinery. miRNAs are RNAs ~22 nucleotides in length that negatively and specifically regulate mRNA expression by binding to complementary sequences on their targets. Hammell's efforts have resulted in identification of a novel, highly conserved family of proteins called TRIM-NHLs that physically associate with the molecular complex (called miRISC) through which miRNAs induce gene silencing. These proteins, together with particular miRNAs, function to regulate animal development. In addition to TRIM-NHL proteins, the team has used classical genetic approaches to identify other regulatory components that tailor miRNA function (via responding to other developmental cues or physiological signals) to specific genetic pathways and/or cell types. Because most of the components identified in these screens are highly conserved, the lab uses model systems including *Drosophila*, mouse, and human to understand how these proteins work at the molecular level.

Leemor Joshua-Tor's lab studies the molecular basis of nucleic acid regulatory processes by using the tools of structural biology and biochemistry to examine proteins and protein complexes associated with these processes. They use X-ray crystallography to obtain three-dimensional structures of individual proteins. Biochemistry and molecular biology enable them to study properties that can be correlated with protein structure and function. It was Joshua-Tor and her team that first obtained the structure of a full-length Argonaute protein, work that instantly solved a long-standing puzzle in the RNAi field. By observing the structure, they realized that Argonaute was the long-sought Slicer, which performs the critical slicing event in RNAi, cleavage of the mRNA. Joshua-Tor is also well known for her work on the helicase enzyme, which acts to unwind DNA strands during the DNA self-replication process.

Adrian Krainer's lab studies the mechanisms of RNA splicing, the ways in which they go awry in disease, and the means by which faulty splicing can be corrected. Their approach has borne fruit in the study of spinal muscular atrophy (SMA), a neuromuscular disease that is the leading genetic cause of death in infants. Their ability to correct an mRNA splicing defect in SMA that makes a gene called *SMN2* only partially functional forms the basis of a potentially powerful therapeutic approach. Last year, the team identified a compound that stimulates SMN protein production by altering RNA splicing. This year, they demonstrated its efficacy. By introducing chemically modified pieces of RNA called antisense oligonucleotides (ASOs) into the spinal cords of mice, they succeeded in reversing symptoms of Type III SMA. The lab's expertise in splicing has also helped to shed light on the abnormal glucose metabolism of cancer cells, sometimes referred to as the Warburg Effect. Krainer and colleagues this year described for the first time how three molecular splicing factors contribute to high levels of PK-M2, an isoform of pyruvate kinase and a key mediator of glucose metabolism in cancer cells.

David L. Spector's lab studies the spatial organization and regulation of gene expression. Their in vivo approach is exemplified in a live cell gene expression system that has made possible the examination in real time of the recruitment of members of gene expression and silencing machineries. A current research focus is the distribution and dynamics of nuclear Polycomb complex proteins, known to keep genes in a silent state. The team seeks to target them to segments of DNA as a means of selectively silencing specific genes. Another focus is the study of long noncoding RNAs (ncRNAs) retained in the nucleus, whose functions are still mostly unknown. This year, Spector and colleagues,

using three-dimensional live cell imaging, discovered that the formation of a type of nuclear sub-compartment called the paraspeckle is triggered by a pair of RNA molecules, which also maintain its structural integrity. The team uncovered this unique structure-building role for the RNAs by keeping a close watch on them from the moment they came into existence within a cell's nucleus. This revealed that when the genes for these RNAs are switched on, and the RNAs are made, they recruit other RNA and protein components and serve as a scaffolding platform upon which the components assemble to form paraspeckles. Paraspeckles have a role in regulating the release of certain protein-coding mRNAs from the cell nucleus.

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

Bruce Stillman's lab studies the process by which DNA is copied within cells before they divide in two. Working with yeast and human cells, Stillman and colleagues have identified many of the cellular proteins that function at the DNA replication fork during the S phase, the portion of the cell division cycle when DNA synthesis occurs. Among these proteins are those that facilitate the assembly of chromatin, the protein–DNA complexes that form the chromosomes. The prime focus of current research, however, is the mechanism that initiates the entire process of DNA replication in eukaryotic cells. At the heart of this mechanism is a protein that binds to "start" sites on the chromosomes, called the origin recognition complex (ORC). Stillman's research has demonstrated that the ORC is also involved in the process of segregating duplicated chromosomes in mitosis. The team has found the ORC at centrosomes and centromeres, structures that orchestrate chromosome separation in mitosis. This year, the team discovered how a protein known to be an essential activator of DNA replication actually triggers this process. They found that DDK phosphorylates a protein called Mcm4, specifically within a domain that acts as a brake to prevent the double helix from being unwound. The phosphorylation releases this brake, thus initiating the replication of unwound DNA strands.

DEVELOPMENTAL REGULATION OF microRNA ACTIVITY

C.M. Hammell   D.M. King

Animal development is remarkably robust and requires the integration of both spatial and temporal genetic programs. Our understanding of the genetic circuitry regulating spatial patterning has illuminated strategies shared by most organisms, but the underpinnings of how temporal gene expression is coordinated in animals remain obscure. The genetic tractability, invariant cell lineage, and ability to observe cell division patterns in a living animal have made *Caenorhabditis elegans* the premier organism to address these questions. Mutations that alter stage-specific developmental programs have revealed conserved classes of heterochronic genes that control the cell fates of the essentially invariant postembryonic cell lineages.

One paradigm that emerged from these studies is that the expression of master regulatory genes required for early temporal developmental programs (usually encoding transcription factors, RNA-binding proteins and other regulatory components) is posttranscriptionally repressed by the activity of specific microRNAs (miRNAs). Although much is known about the transcriptional activation and biochemical maturation of miRNAs, our understanding of how these processes are coordinated to create sharp transitions in gene expression at the molecular and cellular levels is limited.

Genetic Screens in *C. elegans* Identify Components That Regulate miRNA Expression and Ensure Normal Developmental Timing

Under favorable environmental conditions, *C. elegans* larva progress through four larval stages (L1–L4) that are punctuated by molts to a reproductively competent adult within ~40–50 h after hatching. At each of these stages, a variety of cells (including hypodermal, neuronal, muscular, and intestinal cells) execute stage-specific cell division and cell-fate specification programs. The heterochronic genes control these stage-specific events. Mutations of components in this regulatory pathway result in two general types of developmental timing defects. Precocious heterochronic mutants cause stage-specific developmental programs to occur earlier than normal (usually at the consequence of normal programs). In contrast, retarded mutants reiterate stage-specific cell divisions and, as a consequence, retain juvenile characteristics and lack adult-specific organs and features. Importantly, each of these phenotypes is robust, specific, and quantifiable and can be monitored by directly observing cell division patterns in live animals or through surveying the expression of individual genes via green fluorescent protein (GFP)-tagged reporter constructs.

To identify common molecular components that modulate the temporal activity of miRNAs, we screened for extragenic suppressors of mutations effecting essential miRNA processing components or contained mutations in specific miRNAs that are required for normal developmental timing. We initially chose three classes of mutations that separately effect discrete phases of *C. elegans* larval development. The first genetic background that we screened focused on the *lin-4* miRNA that is genetically required after the first larval stage to repress the expression of the LIN-14 transcription factor that functions to specify L1 cell fates. *lin-4* null alleles (*lin-4(e912)*) or point mutants that affect only the mature ~21-nucleotide miRNA (*lin-4(ma161)*) fail to down-regulate LIN-14 expression and, as a consequence of continued expression of LIN-14, reiterate L1-specific cell division programs at all successive larval stages. The second genetic context took advantage of specific point mutations that disrupt the mid-domain of the *C. elegans* miRNA-specific Argonaute, *alg-1(ma192)*, that result in severely retarded heterochronic phenotypes that manifest in the L2 to L3 transition. *alg-1(ma192)* animals fail to down-regulate the HBL-1 transcription factor that is normally mediated by the *let-7* family of miRNAs (miR-48, miR-241, and miR-84) and sequence elements in the *hbl-1* 3′UTR (untranslated region). As the down-regulation of *hbl-1* expression is essential to limit the highly prolific cell divisions of the lateral seam cells, *alg-1(ma192)* animals hyperproliferate their epidermal cell components. The final genetic background for this suppression analysis revolved around the highly conserved regulatory circuit involving the *let-7* miRNA required to posttranscriptionally repress the expression of a critical TRIM-NHL domain protein LIN-41 by binding to se-

quences in the lin-41 3′UTR. Null mutations of *let-7, let-7(mn112)*, or hypomorphic alleles that mutate the conserved sequence of the let-7 mRNA, *let-7(n2585)*, do not down-regulate LIN-41. As a consequence of inappropriate LIN-41 expression, the lateral seam cells fail to terminally differentiate and reiterate larval cell division programs, and animals burst from their vulval structures at the normal L4 to adult transition.

Although each of these mutations cause very specific developmental and cellular phenotypes that manifest at distinct critical stages of larval development, we designed a simple screening strategy that could be adapted for all three screens. All three classes of mutants mentioned above share one common phenotype: They lack normal adult-specific gene regulation, which can be monitored by incorporating a GFP reporter into the screened animals. Specifically, we chose the *col-19* promoter (normally driving the adult-specific collagen gene present on the adult cuticle) to drive a GFP-tagged reporter. The *col-19* promoter is positively regulated by the LIN-29 transcription factor, which is the penultimate gene product of the heterochronic pathway. Each of these types of retarded heterochronic mutants fail to turn on *col-19::GFP*, which is easily observable in a fluorescent dissecting microscope. Suppressors for each mutant would be found by identifying individual animals that could restore *col-19::GFP* expression in the F$_2$ generation. Animals positive for this assay would be further characterized for the correction of the stage-specific cell lineage defects and production of other adult structures that were absent in the initial mutant background.

Characterization of *lin-42* and Its Function in miRNA Metabolism

One would expect that the suppression of each of the above mutants (which effect specific phases of developmental timing) would result in the identification of at least three classes of suppressors. Surprisingly, we were able to identify a single complementation group, represented by multiple alleles in each screen that suppressed all retarded heterochronic phenotypes. Several of these alleles were positionally mapped to the left end of chromosome II. Complementation, rescue with cosmids containing the wild-type gene, and sequencing of

the mutations indicated that all suppressors in this special class were alleles of the *lin-42* gene.

lin-42 encodes a *C. elegans* protein that has striking homology with the *period* (*Per*) genes found in *Drosophila melanogaster*, humans, and mice. *Period* homologs in these animals coordinate gene expression patterns in a circadian (once a day) rhythm that function as essential negative regulatory components of the cellular clock. As clock proteins, *Drosophila*, mouse, and human Per cycle in abundance throughout the day, peaking in the subjective daytime and reaching lowest levels in the evening. Although *C. elegans* is not thought to have a clear, entrainable circadian rhythm, the LIN-42 protein also cycles in abundance; it is most abundant during the intermolt periods and is almost absent during each of the molting cycles. Our molecular analysis of *lin-42* mutants suggests that LIN-42 functions to antagonize the accumulation of mature heterochronic and nonheterochronic miRNAs by dampening their expression at a metabolic step at or upstream of pre-miRNA processing. Notably, LIN-42 and the accumulation of primary miRNA (pri-miRNA) levels are antiphasic to each other, with pri-miRNA abundance peaking during the larval molts (low LIN-42) and undetectable during the intermolt periods (when LIN-42 expression is highest). This complementarity in expression raises the important question of whether LIN-42 controls the accumulation of primary and, therefore, mature miRNA levels. Importantly, the precocious heterochronic phenotypes associated with *lin-42* mutations arise as a consequence of an inappropriate overaccumulation of heterochronic miRNAs during the intermolt periods of larval development. These results suggest that miRNA maturation or activity can be directly coupled to the regulatory machinery that controls developmental timing.

Future Directions

We are currently in the process of using biochemical approaches to identify additional cellular components that function with LIN-42 to modulate miRNA expression and ensure normal developmental timing. These efforts will be combined with experiments in *Drosophila* and mouse cell lines to determine if *Period* expression and activity also control miRNA expression in these contexts.

STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

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

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

Mechanisms of RNAi

T. Schalch, C. Faehnle, C. Kuhn, C. Kuscu, S. Lin, D. Bahel, C. Ashar [in collaboration with G.J. Hannon, R.A. Martienssen, Cold Spring Harbor Laboratory; J. Partridge, St. Jude Children's Research Hospital]

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

During RNAi, long double-stranded RNA (dsRNA) is processed to yield short (~19–31 nucleotides) dsRNAs that trigger the RNAi response. These short RNAs become incorporated into effector complexes called the RNA-induced silencing complex (RISC), where in the mature complexes, a single-stranded RNA (ssRNA), the antisense strand of the original dsRNA, is retained in the complex. This short RNA (small interfering RNAs [siRNAs] or microRNAs [miRNAs]) then acts to guide the RISC complex to its target through base complementarity. The best-characterized pathway, and the one that is predominantly used for gene knockdown tech-

nology, is a posttranscriptional silencing (PTGS) pathway called "slicing." Here the RISC complex is targeted to the mRNA and produces an endonucleolytic cut in the mRNA target, thus preventing gene expression from proceeding. Other RNAi silencing pathways such as translational inhibition and transcriptional gene silencing (TGS) are also mediated through RISC complexes. In all cases, these complexes contain a small ssRNA and an Argonaute protein, which serve to define the RISC complex. In the past few years, we have been studying Argonaute family proteins, their complexes, and their roles in various RNAi silencing pathways.

In the fission yeast *Schizosaccharomyces pombe*, assembly of centrometic heterochromatin requires the RITS complex, which consists of Ago1, Tas3, Chp1, and siRNAs derived from centromeric repeats. Our crystal structure of Chp1's chromodomain in complex with a trimethylated lysine-9 H3 peptide (H3K9me) revealed extensive sites of contact that contribute to Chp1's high-affinity binding. We found that this high-affinity binding is critical for the efficient establishment of centromeric heterochromatin, but preassembled heterochromatin can be maintained when Chp1's affinity for H3K9me is greatly reduced. We continue to study this complex that forms a bridge between the RNAi machinery and chromatin.

The Different Faces of E1: A Replicative Hexameric Helicase

E.J. Enemark, S.-J. Lee

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

unique look into the mechanism of translocation of this molecular machine along DNA.

Papillomaviruses are tumor viruses that cause benign and cancerous lesions in their host. Replication of papillomaviral DNA within a host cell requires the viral E1 protein, a multifunctional protein. E1 initially participates in recognizing a specific replication origin DNA sequence as a dimer with E2, another viral protein. Subsequently, further E1 molecules are assembled at the replication origin until two hexamers are established. These hexamers are the active helicases that operate bidirectionally in the replication of the viral DNA. To unwind DNA, helicases must separate the two strands while moving along, or translocating, on the DNA. On the basis of the structures of the DNA-binding domain of E1 bound to DNA that we determined a few years ago in collaboration with Arne Stenlund's lab, we suggested a mechanism for DNA strand separation. However, the mechanism that couples the ATP cycle to DNA translocation has been unclear.

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

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

ATP molecule, releases one ADP molecule, and translocates one nucleotide of DNA through the interior channel. A full cycle therefore translocates six nucleotides with associated hydrolysis of six ATPs and release of six ADPs. A detailed comparison with other multimeric ATPase motors that highlighted the roles of individual site residues in the ATPase activity was also performed.

We continue to study this helicase and the role that other domains of the protein might have in helicase assembly and activity.

NADP Regulates the Yeast *GAL* Induction System

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

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

To our surprise, we discovered that nicotinamide adenine dinucleotide (NAD) is nestled between Gal80p and Gal4p, making several key interactions with Gal80p. Although NAD appears to be promoting this interaction, we have shown by in vitro pull-down assays that NADP disrupts this interaction. Alterations in the NAD(P)-binding site affects the initial rate of *GAL* induction in vivo, but not overall final expression levels.

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

tively converting NADPH to NADP in the presence of a substrate, causing it to disassociate from Gal4p.

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

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RNA SPLICING

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Mechanisms of Constitutive and Alternative Pre-mRNA Splicing

RNA splicing is required for correct expression of most eukaryotic protein-coding genes. The spliceosome selects authentic splice sites with very high fidelity, relying on limited sequence information present throughout introns and exons. In humans, >75% of genes are expressed via alternative splicing, giving rise to multiple protein isoforms. The choice of alternative splice sites is commonly regulated to alter gene expression, either tissue-specifically or in response to a developmental program or to signaling pathways. The fact that multiple protein isoforms can be expressed from individual genes demonstrates that the classical "one gene–one enzyme" paradigm is no longer valid and provides an explanation for the unexpectedly small number of genes uncovered by genome-sequencing projects.

Both constitutive and alternative splicing mechanisms involve numerous protein components, as well as RNA components that are part of small nuclear ribonucleoprotein (snRNP) particles. The work in our lab focuses on the identification and molecular characterization of protein factors and sequence elements that are necessary for the catalysis and fidelity of splicing and/or for the regulation of alternative splice site selection. We are interested in how the spliceosome correctly identifies the exons on pre-mRNA, and how certain point mutations in either exon or intron sequences cause aberrant splicing, leading to various human genetic diseases. Related areas of interest include the remodeling of mRNP architecture as a consequence of splicing, which influences downstream events, such as nonsense-mediated mRNA decay (NMD); the role of alternative splicing misregulation in cancer; and the development of effective methods to correct defective splicing or modulate alternative splicing, especially in a disease context. A summary of some of our recently published studies is provided below.

Autoregulation and Arginine Methylation of SRSF1

SRSF1 (formerly SF2/ASF) is a prototypical serine- and arginine-rich (SR) protein, with important roles in splicing and other aspects of mRNA metabolism. *SRSF1*, the gene encoding this protein, is a potent proto-oncogene with abnormal expression in many tumors. We found that SRSF1 negatively autoregulates its expression to maintain homeostatic levels. We characterized six alternatively spliced SRSF1 mRNA isoforms: The major isoform encodes full-length protein, whereas the others are either retained in the nucleus or degraded by NMD. Unproductive splicing accounts for only part of the autoregulation, which occurs primarily at the translational level. The effect is specific to SRSF1 and requires RNA recognition motif 2 (RRM2). The ultraconserved 3′-untranslated region (UTR) is necessary and sufficient for down-regulation. SRSF1 overexpression shifts the distribution of target mRNA toward monoribosomes, and translational repression is partly independent of Dicer and a 5′ cap. In collaboration with Jun Zhu (Duke University), miR-7 was also shown to contribute to SRSF1 autoregulation. Thus, multiple posttranscriptional and translational mechanisms are involved in fine-tuning the expression of SRSF1.

Alternative splicing and posttranslational modifications (PTMs) are major sources of protein diversity in eukaryotic proteomes. Functional studies of SR protein PTMs have exclusively focused on reversible phosphorylation of serine residues in their carboxy-terminal RS domain. We confirmed that human SRSF1 is methylated at residues R93, R97, and R109, which were first identified in a global proteomic analysis of arginine methylation. We further investigated whether these methylated residues regulate the properties of SRSF1, which normally shuttles between the nucleus and the cytoplasm. We found that the three arginines additively control the subcellular localization of SRSF1,

and both the positive charge and the methylation state are important. Mutations that block methylation and remove the positive charge result in cytoplasmic accumulation of SRSF1. The consequent decrease in nuclear SRSF1 levels prevents it from modulating alternative splicing of target genes, results in higher splicing-enhancer-dependent translation stimulation, and abrogates the enhancement of nonsense-mediated mRNA decay.

Alternative Splicing and Cancer Cell Metabolism

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

Targeted Antisense Modulation of Alternative Splicing for Therapeutic Purposes

Spinal muscular atrophy (SMA) is a common, autosomal-recessive motor neuron disorder caused by homozygous deletion or mutation of the survival-of-motor-neuron gene, *SMN1*. A closely related *SMN1* paralog, *SMN2*, is present in all patients and differs from *SMN1* by a C to T transition in exon 7 that causes substantial skipping of this exon, such that *SMN2* expresses only low levels of functional protein. *SMN2* decreases the severity of SMA in a copy-number-dependent manner. We continue to explore strategies to increase the extent of exon 7 inclusion during splicing of *SMN2* transcripts, for eventual therapeutic use in SMA. This translational research is being done in collaboration with Frank Bennett and colleagues (Isis Pharmaceuticals), and Marco Passini and colleagues (Genzyme Corporation).

We previously identified a potent $2'$-O-(2-methoxyethyl) (MOE) phosphorothioate-modified antisense oligonucleotide (ASO-10-27) that blocks an *SMN2* splicing silencer element in intron 7 and efficiently promotes exon 7 inclusion in transgenic mouse peripheral tissues after systemic administration. We have now addressed its efficacy in the spinal cord—a prerequisite for disease treatment—and its ability to rescue a mild SMA mouse model that develops tail and ear necrosis, resembling the distal tissue necrosis reported in some SMA infants. Using a micro-osmotic pump, we directly infused the ASO into a lateral cerebral ventricle in adult mice expressing a human *SMN2* transgene; the ASO gave a robust and long-lasting increase in *SMN2* exon 7 inclusion measured at both the mRNA and protein levels in spinal cord motor neurons. A single embryonic or neonatal intracerebroventricular ASO injection strikingly rescued the tail and ear necrosis in this mild model of SMA.

Injection of ASO-10-27 into the cerebral lateral ventricles of neonate mice with a severe form of SMA again resulted in a splicing-mediated increase in SMN protein in the central nervous system (CNS). In addition, this resulted in an increase in the number of motor neurons in the spinal cord, which led to improvements in muscle physiology, motor function, and survival. Intrathecal infusion of ASO-10-27 into cynomolgus monkeys delivered putative therapeutic levels of the oligonucleotide to all regions of the spinal cord. These data demonstrate that CNS-directed ASO therapy is efficacious and that intrathecal infusion may represent a practical route for delivering this therapeutic oligonucleotide in the clinic.

More generally, these experiments demonstrate that ASOs can be used to efficiently redirect alternative splicing of target genes throughout the CNS.

Splice Site Recognition and Human Genetics

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

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CELL BIOLOGY OF THE NUCLEUS

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

The Dynamics of Gene Expression/Repression

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Our research during the past year has focused on the spatial and temporal aspects of gene expression. Transcription is silenced during cell division; however, it must be reactivated upon exit from mitosis to maintain cell lineage and cell cycle progression. The transmission of the gene expression program from mother to daughter cells has been suggested to be mediated by gene bookmarking, which may involve transcription factors, histone modifications/variants, and/or DNA methylation. However, the mechanism by which gene bookmarking mediates rapid postmitotic transcriptional reactivation remains unclear. We used a real-time gene expression system to quantitatively demonstrate that transcriptional activation of the same genetic locus occurs with a significantly more rapid kinetics in postmitotic cells versus interphase cells (Fig. 1). RNA polymerase II large subunit (pol II) and bromodomain protein 4 (BRD4) were recruited to the locus in a different sequential order upon interphase initiation versus postmitotic reactivation resulting from the recognition by BRD4 of increased levels of histone H4 lysine-5 acetylation (H4K5Ac) on the previously acti-
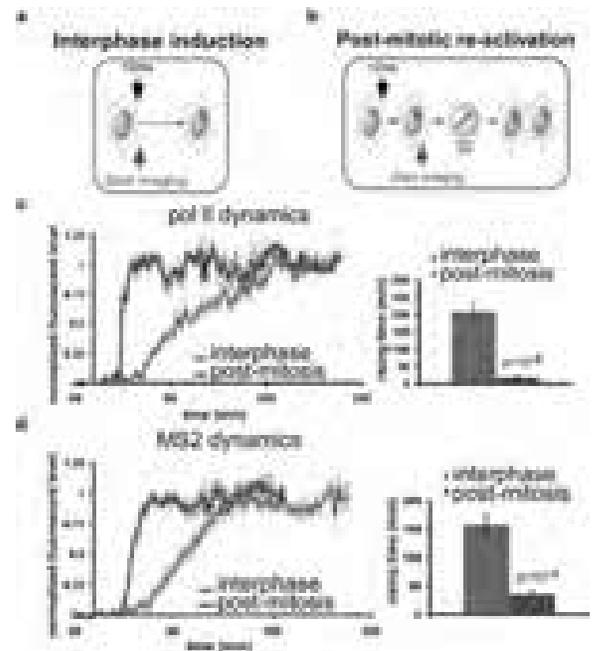
Figure 1. Transcriptional activation of the same locus exhibits faster kinetics during postmitotic activation than in the previous interphase. (*a,b*) Outline of experimental design. After transcriptional induction in interphase (*a*) or postmitosis (*b*), the mCherry-RNA pol II or MS2-YFP (nascent transcripts) fluorescent signals were quantified over time and plotted (*c,d*). For RNA pol II: $n = 17$ for interphase induction, $n = 19$ for postmitotic reactivation. For MS2: $n = 13$ for interphase induction, $n = 25$ for postmitotic reactivation.

vated locus. BRD4 accelerated the dynamics of mRNA synthesis in postmitotic cells by decondensing chromatin and hence facilitating transcriptional reactivation.

Taken together, our results suggest the following model to facilitate rapid postmitotic transcriptional activation. Upon transcriptional induction in interphase, various factors involved in gene expression are recruited to the locus. Transcriptional activation results in an increased level of H4K5Ac at the promoter region of the locus. Upon entry into mitosis, transcription is shut

down due to the condensation of chromatin and loss of the transcription machinery. However, the enhanced level of H4K5Ac is maintained during mitosis and transmitted to daughter cells, serving as a gene bookmark for postmitotic transcriptional reactivation. Upon exit from mitosis, BRD4 is rapidly recruited back to the daughter loci, presumably through recognition of H4K5Ac at the promoter region, and this recruitment leads to rapid chromatin decondensation at the locus, which further facilitates the recruitment of other members of the transcription complex, i.e., pol II and CDK9. BRD4 and other members of the transcription machinery then work cooperatively to achieve the rapid postmitotic transcriptional reactivation of the daughter loci in early G_1 phase cells.

In summary, we established a system to investigate gene bookmarking during postmitotic transcriptional reactivation and its underlying mechanism with integrative approaches. Using a real-time imaging approach, we quantitatively revealed the significantly more rapid kinetics of postmitotic transcriptional reactivation than interphase transcriptional activation at the same genetic locus in individual living cells, demonstrating that postmitotic transcriptional reactivation is mediated by a distinct mechanism mediated by gene bookmarking. Furthermore, tethering experiments revealed a previously unrecognized function of BRD4 in chromatin decondensation, which may be key to the mechanism mediating the rapid kinetics of transcriptional reactivation in postmitotic cells. Together, our findings significantly advance the understanding of gene bookmarking and the mechanism of epigenetic memory in mediating transcriptional reactivation.

Examination of DNA Repair Pathway Choice Upon Zinc Finger Nuclease-Induced Double-Strand Breaks

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In addition to examining the dynamics of transcription, we have also been interested in examining the assembly of the DNA-repair nanomachinery at sites of DNA damage. We have developed and utilized live-cell imaging approaches in order to gain insight into the issues and choices that must be considered by a cell to repair DNA double-strand breaks (DSBs). We have demonstrated the recruitment of the DNA DSB nonhomologous end joining (NHEJ) or homologous recombination (HR) repair nanomachineries to green fluorescent protein (GFP)-

ZFN-induced DSBs, at a multicopy DSB reporter locus in human U2OS 2-6-3 cells. Interestingly, upon expression of both the NHEJ (mCherry-Ku80) and HR (EYFP-Rad51) marker proteins in U2OS 2-6-3 cells, a population of cells exhibited recruitment of members of both machineries, whereas others recruited only HR proteins at the DSBs. This observation prompted us to carry out a quantitative analysis of the frequency of recruitment of NHEJ or HR repair pathway proteins to ZFN-induced DSBs. Such analyses showed that the HR nanomachinery was recruited to the DSBs in 90% of the asynchronously dividing U2OS 2-6-3 cells, whereas the NHEJ proteins were recruited in 66% of the cells. However, the recruitment of both the NHEJ and HR machineries may be the consequence of the 200-copy reporter locus.

To achieve our goal of visualizing the repair process of a single DSB in living cells by the NHEJ/HR machinery, we have established a stable U2OS-pSOEP-62L cell line carrying one to two copies of a DSB reporter locus. In this cell line, we have demonstrated the recruitment of mCherry-53BP1 to GFP-ZFN-induced DSB at a single site in the human genome. Imaging (at 0.1% laser intensity and 5-ms exposure) of the LacI-enhanced yellow fluorescent protein (EYFP)-labeled locus in U2OS-pSOEP-62L followed by postacquisition processing by a denoising algorithm allows for live-cell data to be acquired at 100 times lower light exposure. This is especially critical, during fast image acquisition of dynamic events, at very high temporal resolution in a defined window of time in living cells. In addition, we have also optimized imaging (without considerable photobleaching) the LacI-EYFP-labeled one-to-two copy locus (containing ~5 × 10^2 to 1 × 10^3 FPs) in U2OS-pSOEP-62L cells using the OMX microscope in structured illumination mode at 100-nm super-resolution. We are in the process of making additional stable cell lines that will express multiple DSB-repair components in a stable, inducible and tightly regulatable way. We have also initiated optimization of live-cell imaging in the one-to-two DSB reporter cell line. We followed the dynamic disassembly of the DSB-repair nanomachinary (mCherry-53BP1) at the one-to-two GFP-ZFN-induced DSBs, every 10 min for a period of 5 h. Ongoing studies are examining the precise timing of the DSB-repair machinery disassembly. Ultimately, by following living cells stably expressing various fluorescent protein markers through a complete cell cycle, we will be able to distinguish (based on cell size and morphology) the G_1, G_2, and M stages of the cell cycle and correlate this to the recruitment of NHEJ/HR proteins.

Characterization of a Nuclear-Retained Noncoding RNA Involved in the Assembly of a Nuclear Body

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The cell nucleus is a highly compartmentalized organelle harboring a variety of dynamic membraneless nuclear bodies. How these subnuclear domains are established and maintained is not well understood. During the past year, we have continued our analysis of the role of long nuclear retained noncoding RNAs (ncRNAs) in nuclear organization. We have focused our efforts on the MEN ε/β locus, which is up-regulated 3.3-fold upon myoblast differentiation to myotubes. Two ncRNA isoforms are produced from a single pol II promoter, differing in the location of their 3′ ends. MEN ε is a 3.2-kb polyadenylated RNA, whereas MEN β is a ~20-kb transcript containing a genomically encoded poly(A)-rich tract at its 3′ end. These RNA transcripts are broadly expressed in adult mouse tissues and conserved among mammals. The MEN ε/β transcripts are localized to nuclear paraspeckles and directly interact with p54/nrb. Knockdown of MEN ε/β expression results in the disruption of nuclear paraspeckles. During the past year, we investigated the molecular mechanism of how paraspeckles are assembled and organized. Paraspeckles are discrete ribonucleoprotein bodies found in mammalian cells and implicated in nuclear retention of hyperedited mRNAs. We developed a live-cell imaging system that allows for the inducible transcription of MEN ε/β ncRNAs and the direct visualization of the recruitment of paraspeckle proteins. The reporter, modified from our previous studies and integrated at a single site in the C2C12 genome, is visualized by expression and binding of enhanced cyan fluorescent protein (ECFP)-LacI at the LacO array. Upon doxycycline (DOX) induction, a minimal cytomegalovirus (CMV) promoter drives expression of MEN ε/β ncRNAs tagged with 24 MS2 RNA stem-loop repeats. Nascent MEN ε/β transcripts are visualized by accumulation of EYFP-tagged MS2 viral coat protein, which binds the MS2 repeats within the transcripts. This system allows for the MEN ε/β ncRNAs transcription site, nascent MEN ε/β ncRNAs tran-scripts, and protein recruitment to be simultaneously visualized in single living cells. Using this system, we were able to demonstrate that MEN ε/β ncRNAs are essential to initiate the de novo assembly of paraspeckles. These newly formed structures effectively harbor nuclear retained mRNAs, confirming that they are bona fide functional paraspeckles. By three independent approaches, we showed that it is the act of MEN ε/β transcription, but not the ncRNAs alone, that regulates paraspeckle maintenance. Finally, fluorescence recovery after photobleaching analyses supported a critical structural role of MEN ε/β ncRNAs in paraspeckle organization. Together, our findings established a model in which MEN ε/β ncRNAs serve as a platform to recruit proteins to assemble paraspeckles. These data established a seeding model for nuclear body formation in which MEN ε/β ncRNAs were identified as pivotal nucleating molecules, driven by transcription, to recruit proteins to assemble paraspeckles. Ongoing studies are examining the function of MEN ε/β RNAs in differentiation.

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MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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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 associated with initiation of DNA replication. We are studying the biochemical properties of the viral E1 and E2 proteins and how these two proteins interact with the viral origin of DNA replication and with the cellular replication machinery to generate initiation complexes. Our studies demonstrate that the E1 protein has all of the character-

istics 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 and the initial opening of the DNA duplex (melting), as well as 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.

A Sequestration Module in the Amino Terminus of E1 Controls Access to the Region Containing the Nuclear Localization Signal

The papillomavirus E1 protein is a multifunctional protein, which carries out essential functions in viral DNA replication. Although E1 is well-studied, functions have not been assigned to all parts of the protein. The function of the amino-terminal ~150 residues has especially remained mysterious. Although a part of this sequence is involved in nuclear import and export, these sequences account for only a fraction on the amino-terminal domain. Given the parsimony that usually characterizes viral proteins, it is likely that additional functions are present in the amino-terminal domain. It has previously been demonstrated that a peptide derived from the E1 nuclear localization signal (NLS) had DNA-binding activity. We wanted to determine whether we could detect this DNA-binding activity in the context of the larger E1 protein. Because full-length E1 encodes one DNA-binding activity in the E1 DNA-binding do-

main (DBD) and another DNA-binding activity in the helicase domain, detection of this additional DNA-binding activity in the context of the full-length E1 would be difficult. We therefore focused on just the amino-terminal domain. We could isolate a minimal fragment containing residues 92–120 that had strong DNA-binding activity. Surprisingly, however, larger fragments had reduced DNA-binding activity or lacked DNA-binding activity altogether. Indeed, we could observe a distinct pattern. The intact amino-terminal fragment ($E1_{1-159}$) lacked DNA-binding activity altogether. Deletion of 25, 35, or 45 residues from the amino terminus gradually increased the DNA-binding activity of the fragment. Similarly, deletion of 30 residues from the $E1_{1-159}$ fragment resulted in robust DNA-binding activity. These results indicate that in the presence of 30–40 residues from the amino terminus and 30–40 residues from the carboxyl terminus of the $E1_{1-159}$ fragment, the DNA-binding activity is hidden or sequestered. Removal of either the amino-terminal or carboxy-terminal residues results in strong DNA binding. To determine the function of this sequestered DNA-binding activity, we generated mutations in the minimal fragment with DNA-binding activity and tested for DNA binding. We identified one point mutation, R106A, that failed to bind DNA. Interestingly, when introduced into the viral genome, this mutation resulted in a severe transformation defect, indicating either that the mutation affects nuclear localization or that the DNA-binding activity has a role in the viral life cycle. To establish whether the amino-terminal domain in E1 has a role in the biochemical activity of the E1 protein, we expressed and purified full-length E1 containing several of the amino-terminal deletions described above. Interestingly, although a large deletion mutant, which removes most of the amino-terminal domain, was fully functional for cell-free DNA replication, several smaller deletions lacked DNA replication activity in the cell-free system. On the basis of our biochemical assays, these mutants are defective for the formation of a specific E1 double trimer complex, which serves to generate local melting at the viral origin of DNA replication. These results indicate that the amino-terminal domain most likely has a regulatory role in viral DNA replication and is not essential for the initiation process. The role of the sequestration module still remains to be determined.

A Mechanism for Local Melting

Preparation of DNA templates for replication requires the initial opening of the duplex to expose single-stranded DNA (ssDNA). This locally melted region is required for replicative DNA helicases to initiate unwinding. How local melting is generated in any replicon is unknown, but initiator proteins from some viruses, including the BPV E1 protein, can perform this process. E1 is therefore an excellent tool and can provide understanding of the local melting process. Incubation of E1 with an ori fragment in the presence of ATP gives rise to an E1 double trimer (DT) complex, which slowly is converted into an E1 double hexamer (DH) complex. In the process, the ori DNA is unwound as demonstrated by the appearance of ssDNA in the form of an ssDNA/SSB (single-stranded DNA-binding protein) complex.

To study the local melting process, we generated a complete set of surface mutations in the E1 helicase domain based on the available structural information and performed a screen for mutations that were capable of forming the E1 DT but that failed to form the E1 DH. Of ~70 surface mutants, we identified 10 that were defective for the transition between the DT and the DH. These ten mutants were tested for local melting in permanganate reactivity assays. Nine of the mutants arrested the melting process at the same time, within the first 2 min of melting, and one mutant arrested the melting at ~10 min. Interestingly, when we determined where these residues were located in the E1 hexameric ring structure, we found that the nine mutants in the group that arrested early all fell neatly in the interface between adjacent helicase domains in the hexamer. The tenth mutant was located on the inner surface of the hexameric ring. Because the interface mutants all have wild-type DNA helicase activity, which requires hexamer formation, these mutants were not defective for hexamer formation per se. Instead, the defect of the interface mutants is in local melting, which is a prerequisite for DH formation.

These results indicated to us that the E1–E1 interface in the E1 hexamer was important for local melting but not for hexamer formation. Because the hexamer is held together by interactions between the oligomerization domains, it is likely that the helicase domain interface is involved in generating the force required for local melting. On the basis of these results, we wanted to test a model where the DT generates local melting by untwisting the ori DNA. Because such a mechanism would depend on force being transmitted through the double-stranded DNA (dsDNA), we could test this idea by generating ori templates containing nicks. Interestingly, when nicks were present between the attachment sites for the E1 DT, melting could not be observed, indicating that intact dsDNA is required for local melting, con-

sistent with an untwisting mechanism. In contrast, when nicks were introduced outside the E1 DT attachment sites, no effect on local melting could be observed. These results make it possible for the first time to propose a mechanistic model for local melting. Furthermore, although the structure of the interface between the E1 monomers in the DT is not known, the mutational screen maps the interface. The sensitivity of the DT-to-DH transition to single-amino-acid substitutions demonstrates that this interface is very sensitive to disruption, and because it is also well-conserved, it makes this interface a potential target for small molecules that could interfere with viral replication.

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Stephen Schuck

DNA REPLICATION AND CHROMATIN INHERITANCE

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Our genome contains the genetic information that is transmitted from one cell to its two daughter cells during cell proliferation and development of tissues and organs. The genetic information in DNA is also copied, and half of the duplicated information is passed on from parent to child. The process of duplicating the DNA double helix—the process that is central to genome inheritance—is therefore of fundamental importance and has been the focus of research in this laboratory for 30 years at Cold Spring Harbor. DNA is wrapped up with proteins that form chromatin, and chromatin structures, which happen to be different in the various cell types in our body, are also transmitted from one cell generation to the next during the process that yields ~100 trillion cells in our adult body. Thus, the information transfer must be accurate and efficient. This year, progress has been made on understanding how the process of genome duplication is controlled, and we have gained additional insight into the mechanism of chromatin inheritance and higher-order chromosome structure.

Inheritance of Chromatin and Gene Silencing

The genome of cells contains the DNA double helix that is wrapped with histone proteins to form nucleosomes, the fundamental building blocks for chromatin organization. Each nucleosome consists of two copies of a dimer of histones H3 and H4 and two copies of a dimer of H2A and H2B, the eight proteins forming a core protein structure around which ~140 bp of DNA is wound. Strings of nucleosomes make up our entire genome, and gaining access to the DNA in the nucleosomes has a major role in controlling access to DNA regulatory regions by proteins that control gene transcription. The differentiated patterns of gene expression that are inherited from one generation to the next in a particular developmental cell lineage are often referred to as epigenetic inheritance. Every cell gets the same DNA sequences, but modifications of DNA, via DNA methylation, and modifications on the histones by both methylation and acetylation, as well as other modifications, set up heritable differences in gene expression patterns.

In the mid 1980s, we developed a biochemical system that could assemble nucleosomes on DNA during DNA replication and this process has been studied ever since. A key player in this process is the chromatin assembly factor-1 (CAF-1) that binds histone H3-H4 dimers and loads two dimers onto replicating DNA, followed by loading two dimers of H2A-H2B to form the nucleosomes with DNA wrapped around the outside of the eight-histone protein core. CAF-1 was shown to bind to a key DNA replication protein, PCNA (proliferating cell nuclear antigen), that also functions as a DNA polymerase clamp, which tethers the DNA polymerase to the replicating DNA and recruits many proteins to the replicating DNA. We have shown that certain mutations in PCNA and CAF-1 cause a defect in inheritance of chromatin during DNA replication in both yeast and human cells. Indeed, CAF-1 is essential for human cell proliferation, but in yeast, it causes subtle effects on chromatin structure and function.

Telomeres, the very ends of chromosomes, have long been known to influence the expression of genes that lie in their vicinity, and, in particular, they cause expression of a gene in some cells and shut off expression in other genetically identical cells. This epigenetic state is often referred to as telomere position-effect variegation (TPEV). Such an epigenetic state of gene expression near telomeres was first observed in *Drosophila,* but it is known to be widespread and even occurring in human cells. A common assay for inheritance of the epigenetic state of gene expression in chromatin involves the analysis of a *URA3* marker gene that is placed near one telomere on chromosome VII in the yeast *Saccharomyces cerevisiae.* The genetically tractable yeast thus offers a potentially powerful system of understanding how telomeric gene silencing occurs. Research by other investigators in the 1990s established that the *URA3* gene placed at telomere VII-L (*URA3*-TELVII-L) is expressed in some cells in the population and thus the cells can grow on medium

lacking uracil; however, in other cells in the same population, the *URA3* gene is shut off, causing cells to be resistant to the toxic drug 5-fluoroorotic acid (5-FOA) that the Ura3 enzyme metabolizes. This situation creates an epigenetic state of expression of the *URA3* gene near this telomere, or TPEV.

We demonstrated a number of years ago that mutations in the DNA replication protein PCNA, encoded by the *POL30* gene and its partner CAF-1, are required for inheritance of the silenced state of *URA3*-TELVII-L and thus the yeast could not maintain a 5-FOA-resistant phenotype. PCNA and CAF-1 cooperate to load histones H3-H4 onto DNA for assembly of nucleosomes during DNA replication. We used a genetic screen to identify additional proteins that might function in this pathway and obtained some surprising results that resulted in a reassessment of a large amount of literature on telomeric silencing in yeast.

For three proteins implicated in TPEV, CAF-1, PCNA, and the histone methyltransferase DOT1, our results demonstrated that the TPEV phenotype is due either to alterations in nucleotide metabolism or to specific promoter effects at that particular locus on chromosome VLL and not due to heterochromatin formation, which was widely assumed. Specifically, the locus into which the *URA3* gene was introduced just happened to be a particularly sensitive locus for TPEV because the *URA3* gene was expressed at low levels; however, it was induced when the drug 5-FOA was added to cells. The increased Ura3 enzyme increased the flux of the toxic 5-FOA into nucleotide precursors of DNA replication via the enzymes ribonucleotide reductase and thymidylate synthase. Thus, the very drug used to measure the epigenetic state induced changes in *URA3* gene expression, and thus, the phenotype of the cells was altered because of metabolic changes and not changes in gene silencing. These 5-FOA-induced changes in metabolism occurred more readily in *pol30-8, cacΔ,* and *dot1Δ* mutants, thereby inappropriately assigning them as modulators of TPEV. Although we agreed that SIR proteins do silence gene expression at this telomere, we suggested that the results of many other mutant studies implicating a wide variety of genes in TPEV could be explained not by defects in epigenetic inheritance but by other effects of altered nucleotide metabolism.

Our research, however, did discover what is most likely the real defect in DNA replication-coupled assembly by the CAF-1–PCNA pathway in yeast, namely, a defect in global histone H3-H4 density, and hence nucleosome density on chromosomes, that affects the expression of genes normally not expressed or poorly expressed in the haploid cells we studied. This observation is interesting because CAF-1 is essential in human cells and binds histone H3.1 but not histone H3.3, yet CAF-1 is not essential in yeast and yeast have only histone H3.3. Thus, the H3.1-CAF-1 axis is essential for propagation of the genomes of cells with large genomes. Moreover, CAF-1 defects in plants cause profound variegation among individuals (stem fasciation), and we suggest that this is caused by a similar, global defect in histone deposition that has major secondary consequences for gene expression.

Break-Induced and DNA Replication Factors

In last year's report, we described results of an analysis of the essential nature of the Cdc7-Cbf4 (DDK) protein kinase in DNA replication. We demonstrated that the essential target for DDK is a domain in the Mcm4 protein that acts as an intrinsic inhibitor of the initiation of DNA replication. We collaborated with the lab of Jim Haber at Brandeis University to study the role of DDK in double-stranded DNA break-induced replication (BIR). This replication-associated repair pathway is an efficient homologous recombination pathway used to repair a DNA double-strand break when homology is restricted to one end of a chromosome. The Haber lab has shown that all three major replicative DNA polymerases are required for BIR, including the otherwise nonessential Pol32 subunit of DNA polymerase δ. BIR also requires the replicative DNA helicase (Cdc45, the GINS, and Mcm2-7 proteins) as well as Cdt1. In contrast, both subunits of the origin recognition complex (ORC) and Cdc6, which are required to create a prereplication complex (pre-RC), are dispensable. In this collaboration, we showed that the Cdc7 kinase, required for both initiation of DNA replication and postreplication repair, is also required for BIR. These results suggest that origin-independent BIR involves cross-talk between normal DNA replication factors and post-replication repair and that DNA replication during BIR involves many of the key replication proteins that are required to replicate the DNA during S phase.

Origin Recognition Complex and Heterochromatin

A number of years ago, Mike Botchan and his colleagues showed that *Drosophila* ORC localized with

heterochromatin protein 1 (HP1) and that the Orc1 subunit interacted with this protein. Prior work from this laboratory demonstrated a similar interaction in human cells; however, in the human cell system, there was a paradox because Orc1 is degraded at the G_1-to-S-phase transition and yet HP1 proteins still colocalize with Orc2 and Orc3 subunits during S and G_2 phase when there is no Orc1 in the cell. This prompted us to investigate the interactions between HP1 and ORC in more detail.

HP1, which exists in human cells in a number of isoforms (α, β, and γ), contains both chromo- and chromo-shadow domains and localizes to all heterochromatic loci in animal cells, including centromeres. It particularly localizes to heterochromatin marked with the histone K9–trimethylation modification. Surprisingly, it was shown by a number of groups a few years ago that the HP1 proteins were dynamically associated with the heterochromatin, with an apparent half-life on the chromatin of ~4 seconds. We have now demonstrated that in human cells, multiple ORC subunits associate with HP1α- and HP1β-containing heterochromatic foci. Fluorescent bleaching studies indicated that multiple subcomplexes of ORC existed at heterochromatin, with Orc1 stably associated with heterochromatin in G_1 phase, whereas other ORC subunits have transient interactions throughout the entire cell division cycle. In addition to Orc1, we demonstrated that Orc3 directly bound to HP1α and two domains of Orc3, a coiled-coil domain and a separate MIR domain, that is also present in CAF-1 independently bound to HP1α. However, both domains were essential for in vivo localization of Orc3 to heterochromatic foci. Direct binding of both Orc1 and Orc3 to HP1 suggests that following the degradation of Orc1 at the G_1/S boundary, Orc3 facilitates assembly of ORC/HP1 proteins to chromatin. Although depletion of Orc2 and Orc3 subunits by small interfering RNA (siRNA) caused loss of HP1α association to heterochromatin, loss of Orc1 and Orc5 caused aberrant HP1α distribution only to pericentric hetero-

chromatin surrounding nucleoli. Depletion of HP1α from human cells also showed loss of Orc2 binding to heterochromatin, suggesting that ORC and HP1 proteins are mutually required for each other to bind to heterochromatin. Similar to HP1α-depleted cells, Orc2 and Orc3 siRNA-treated cells also showed loss of condensation at α-satellite repeats, suggesting that ORC together with HP1 proteins may be involved in organizing higher-order chromatin structure and centromere function. More recent studies, to be published later, have demonstrated that Orc2 and Orc3 have a direct role in centromere activity in human cells.

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CANCER: GENETICS

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

Gregory Hannon is a pioneer in the study of RNA interference (RNAi), a process in which double-stranded RNA molecules induce gene silencing. Hannon and colleagues have elucidated key elements of the RNAi machinery and have led the way in using RNAi to study cancer biology and genetics, generating a library of short hairpin RNAs (shRNAs) that have been widely applied in gene silencing studies. This year, by analyzing an miRNA molecule produced in developing red blood cells, Hannon's team discovered an alternative pathway of miRNA generation. Existence of this alternative pathway helps to explain evolutionary pressure to maintain a catalytically active Argonaute protein in animal cells. In other work, the Hannon lab discovered that mRNAs can be targeted for destruction by several modes and molecules, highlighting a previously unanticipated complexity in the control and regulation of the cell's genetic messages. Also in 2010, innovative sequencing technologies developed in recent years by Hannon and colleagues were brought to bear in the Neanderthal genome project. The array-capture resequencing method enabled the team to show the remarkable similarity in proteins encoded by corresponding sets of 14,000 Neanderthal and human genes. Exome resequencing also enabled the team to quickly and economically identify a point mutation that causes Joubert syndrome, a rare and devastating neurological illness that disproportionately afflicts Ashkenazi Jews.

Scott Lowe's laboratory studies cancer gene networks and determines how genetic lesions affecting these networks contribute to tumor development and resistance to therapy. This year, Lowe and colleagues showed how mutations in K-*ras* and *p53* act to reinforce one another to change the character of blood precursor cells, transforming them into cells that can renew themselves—and thus proliferate—indefinitely, somewhat as cancer stem cells are theorized to do. Lowe's team has adapted RNA interference (RNAi) technology to produce animal models in which genes can be switched on and off in a spatial, temporal, and reversible manner and has used this to study the impact of tumor suppressor genes on tumor development and tumor maintenance. He also has spearheaded a collaborative effort involving four other CSHL labs to identify novel tumor suppressor genes. He combined his earlier integrated oncogenomics approach on human liver cancers with RNAi-based screening in a mouse model. The lab also studies cellular senescence, a potentially powerful mechanism for suppressing tumor formation. This year, they discovered that the gene-suppressing activity of the retinoblastoma (*Rb*) gene can be traced to its ability to trigger senescence. The team had previously demonstrated that senescence also helps to limit wound-healing responses in liver disease, a finding that suggests that growth arrest has functional relevance, in addition to cancer, in the maintenance of tissue homeostasis following damage.

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

Alea Mills is studying genetic pathways important in cancer and aging, identifying the genetic players and determining how aberrations in their functions culminate in human disease. Through innovative use of a technique called chromosome engineering, the Mills group identified a tumor suppressor gene that had eluded investigators for three decades. The gene, called *Chd5*, was shown

by Mills to regulate an extensive cancer-preventing network. The epigenetic role of *Chd5* in development, cancer, and stem cell maintenance is currently being investigated. The Mills lab is also studying p63 proteins, which regulate development, tumorigenesis, cellular senescence, and aging, in vivo. 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. During the last year, they discovered that a different version of p63, called ΔNp63, reprograms stem cells of the skin to cause carcinoma development—the most prevalent form of human cancer. Modulation of these proteins may offer new ways to treat human malignancies in the future.

Scott Powers' work focuses on gene mutations that cause cancer and factors that influence responses to specific anticancer drugs. His lab uses technologies that probe the entire genome to identify candidate cancer genes and evaluate their functional role in cell transformation and tumor biology. They also use whole-genome technologies to guide development of novel cancer diagnostics and therapeutics. Using DNA copy-number analysis, the Powers group pinpoints novel amplified oncogenes and then applies functional studies to address the validity of candidate genes and the mechanisms by which they are implicated in oncogenesis. They have successfully applied this approach in liver, colon, and lung cancer. Powers has also had an important role in the development of a distinctive CSHL approach to functional study of cancer genes. Called integrative oncogenomics, the approach uses 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. As a follow-on study to last year's discovery of 13 previously unknown tumor suppressors whose deletion resulted in aggressive cancers, this year Powers, together with the Lowe lab, discovered 10 new amplified oncogenes. This is now the basis for a new targeted therapeutic strategy for treating liver cancer.

Michael Wigler's group uses methods for comparative genome analysis to study cancer (together with Jim Hicks of CSHL) and human genetic disorders. These methods evolved from earlier techniques—from representational difference analysis (RDA), used to find tumor suppressors, oncogenes, and pathogenic viruses, to microarray and now to DNA sequence-counting methods, to reveal changes in the numbers of copies of sections of the genome (regions of deletion and duplication), mutations that may underlie the evolution of cancers and human genetic disorders. Wigler's group focuses on breast cancer and leukemias and is engaged in collaborative clinical studies with major national and international research oncologists to discover mutation patterns predicting treatment response and outcome. The group has developed methods for the analysis of the genomes of single cells that have led to new insights into the clonal evolution and heterogeneity of cancers. This work may lead to a better understanding of metastasis and progression, shed light on the stem cell hypothesis of cancer, and have clinical application in the early detection of cancer and its recurrence. Wigler and colleagues made great headway in the discovery of the causative mutations in autism. Their results show that spontaneous mutation has a far greater role in autism than previously suspected. They have developed a new theory of autism's genetic basis that explains otherwise bewildering patterns of inheritance, and they are testing the new genetic model in other disabling genetic disorders, such as congenital heart disease (with Dorothy Warburton at Columbia University) and pediatric cancer (with Ken Offit at Memorial Sloan-Kettering Cancer Center). Wigler also has spearheaded the development of a Center for Quantitative Biology at CSHL, with initial funding from the Simons and Starr Foundations.

RNA INTERFERENCE MECHANISMS AND APPLICATIONS

G. Hannon

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A. Canela	S. Fenoglio	E. Hodges	A. Molaro	F. Rollins	Y. Xu
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Our lab continues to focus on three distinct areas. First, we study RNA biology, with a focus on noncoding RNAs. Second, we study the roles of small RNAs in cancer, mainly breast cancer, and use these small RNAs as tools to uncover tumor-specific vulnerabilities as potential therapeutic targets. Third, we develop technologies, mainly in the areas of mammalian genetics and genomics, with a particular focus on next-generation sequencing. A selected set of laboratory projects are described in detail below.

This year, three students obtained their Ph.D. degrees and departed the laboratory. Ikuko Hotta joined Jim Manley at Columbia and Colin Malone departed to Ruth Lehman's lab at New York University, both as postdoctoral fellows. Yaniv Erlich became a Whitehead fellow at the Massachusetts Institute of Technology. After a brief postdoctoral stint, Adam Rosebrock accepted a position as a fellow at the University of Toronto. Ania Jankowska, who originally joined the lab as an undergraduate volunteer from Stony Brook University, left her technician position at CSHL to enter a Ph.D. program at Columbia University. Finally, Pramod Thekkat moved from CSHL to Novartis.

The lab was joined by four outstanding students. Xin Zhou came from Stony Brook University, whereas Wee Siong Goh (aka Sho), Silvia Fenoglio, and Kaja Wasik came from the Watson School. We were also joined by three new postdoctoral fellows. Sung Wook Chi came from the Darnell lab at The Rockefeller University, and Yang Yu joined us from Case Western Reserve. Stephanie Shaw, who completed her Ph.D. with Adrian Krainer, came back to CSHL after working for a time with Rui Ming Xu.

With the expansion of several large-scale projects, we were joined by a number of new research associates, including Michael Baer, Emily Harrison, Christine Peterson, and Kui Tian, who left recently to take a position in industry.

We continued our tradition of hosting visiting investigators, with Tasman Daish coming to work on mono-treme small RNAs, and two summer undergraduates, Connie Martin and Ashley Maceli having worked with Felix Muerdter and Emily Hodges, respectively.

Genome-Wide Analysis of DNA Methylation During Stem Cell Differentiation and Development

E. Hodges, A. Molaro [in collaboration with A. Smith, University of California, Los Angeles]

DNA methylation is a stable, mitotically inherited epigenetic mark that contributes to the transcriptional programming underlying cellular identity and specification. During the last year, we produced and extensively analyzed genome-wide maps of DNA methylation at single-base resolution for the methylomes of three specific cell types purified from the hematopoietic lineage: an early stem cell and two derived, terminally differentiated cell types of the myeloid and lymphoid branches. Through this project, we have generated the first sequenced methylomes derived from purified human cell populations, as well as the first to be sequenced exclusively from female individuals. Examination of the relationships between the three cell types chosen for this project revealed several interesting findings, including dynamic patterns of expansion and contraction of domains of hypomethylation that correlated significantly with changes in gene transcription. Aberrant DNA methylation leads to deficiencies in stem cell self-renewal and can ultimately disrupt the normal development of mature tissues. In addition, abnormal levels of DNA methylation near the loci of tumor suppressors and oncogenes have been shown to contribute to tumor progression. Therefore, establishing the reference sequence for the methylomes of normal cell types is critical for understanding the changes that contribute to disease. Furthermore, these data will provide insight into differential methylation patterns that influence lineage decisions.

Structural Variation in Human Cancer

A. Canela

Structural variations including copy-number polymorphisms (CNV), as well as insertions, inversions, and translocations, have an important role in tumor development. Although the mutagenic effects of translocations have long been seen as critical to the development of hematological malignancies, only recently have technological advances allowed genome-scale analysis of rearrangements in common solid tumors. We study structural variation in breast cancer cells at single-base resolution by next-generation sequencing. As a first approach, we focused on regions previously characterized by comparative genomic hybridization (CGH) as containing CNVs as landmarks for identifying complex chromosomal rearrangements. We purified these regions in breast cancer cell lines by array-based genomic capture and deciphered them by sequencing the precise structure of genomic rearrangements, which leads to detection as copy-number loss and gain in the CGH (in collaboration with the Wigler lab at CSHL). Because not all the areas of CNV may contain complex chromosomal rearrangements, we used as an unbiased approach large-fragment genomic libraries as "jumping libraries" to scan by sequencing the whole genome for structural variation events. The use of these approaches in a panel of breast cancer cell lines allows us not only to characterize structural variation and study their repercussion in breast tumorigenesis, but also to identify areas of the genome that act as hot spots of structural variation and their potential as landmarks for chromosomal rearrangements in cancer.

Investigating DNA Methylation Profiles during Mammalian Gametogenesis

A. Molaro, E. Hodges, K. Marran [in collaboration with A. Smith, University of California, Los Angeles]

We profiled genome-wide CpG methylation in male germ cells at various stages of gametogenesis. Using high-throughput bisulfite sequencing, we generated the complete map of methylation marks in primary sperm cells from humans and chimpanzees and compared these with somatic cultured cells (Lister et al., *Nature 462:* 315 [2009]). We showed that despite ~70% of all CpGs being methylated, mature germ cells transmit a large number of hypomethylated regions (HMRs) overlapping all genomic annotations. Among these, we characterized an unprecedented set of repeat copies (including retrotransposons) as evading de novo methylation in a germ-cell-specific fashion. We also investigated the methylation status of CpG islands in mature sperm and showed that hypomethylation of these regions was not prerequisite for their maintenance during evolution because many CGIs remain methylated in mature germ cells. In general, sequence conservation correlated well with conservation in methylation status; however, regions of divergence exist. We consequently quantified the impact of CpG variation on methylation in regions of otherwise high sequence conservation and showed that a certain level of decoupling exists between genome and epigenome evolution.

Using Oct4-GFP transgenic mice, we sorted and analyzed the methylation of 13.5-dpc embryonic gonad and showed that only 4% of all CpGs retained methylation at this stage. We also analyzed the methylation profiles of mouse 2-day spermatocytes and saw similar patterns than observed those with human and chimp samples.

We also performed a detailed characterization of methylation as well as transcriptional patterns at several stages of germ cell maturation in wild-type and Piwi-interacting RNA (piRNA)-deficient animals. We found that despite the significant number of retrotransposons potentially targeted by piRNAs, only a small subset depend on the pathway for proper remethylation as the overall methylation levels only drop by 10%. We showed that these elements display a strong transcriptional reactivation prior to their silencing, indicating that they might help their selective targeting by overloading Piwi proteins by displaying nascent transcripts. We propose that whereas most remethylation is established by default as germ cells mature, some retrotransposons mimic regions normally protected (e.g. promoters) and require a transcription-dependent piRNA-mediated pathway to be properly targeted by the de novo methylation machinery.

Probing the Initiation and Effector Phases of the Somatic piRNA Pathway in *Drosophila*

A. Haase, S. Fenoglio, F. Meurdter, P. Guzzardo, B. Czech, A. Gordon, D. Pappin [in collaboration with C. Chen, Applied Biosystems]

Combining RNA interference (RNAi) in cultured cells and analysis of mutant animals, we probed the roles of known piRNA pathway components in the initiation and effector phases of transposon silencing. Squash as-

sociated physically with Piwi, and reductions in its expression led to modest transposon derepression without effects on piRNAs, consistent with an effector role. Alterations in Zucchini or Armitage reduced both Piwi protein and piRNAs, indicating functions in the formation of a stable Piwi RISC (RNA-induced silencing complex). Notably, loss of Zucchini or mutations within its catalytic domain led to accumulation of unprocessed precursor transcripts from flamenco, consistent with a role for this putative nuclease in piRNA biogenesis.

Functional Dissection of Primary piRNA Biogenesis in *Drosophila*

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

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

In *Drosophila melanogaster*, primary piRNA biogenesis involves the processing of a primary transcript into mature small RNAs by so far unknown factors. To selectively search for enzymes involved in such biogenesis or downstream effector pathways, we make use of an insect cell line derived from the ovary of *Drosophila*. This cell line, the ovarian somatic sheet (OSS) cell line, only expresses Piwi but not Aubergine or Argonaute3. Consequently, these cells do not show signatures of secondary piRNA biogenesis or the ping-pong cycle.

We are preparing a genome-wide high-throughput RNAi screen in OSS cells. So far, this included establishing the cell line, testing our capability to transfect and knock down endogenous genes, and ultimately developing an assay to screen for transposon derepression upon disruption of primary piRNA pathways. By testing each one of the ~16,500 double-stranded RNAs in our *Drosophila* RNAi library in individual transfections, we are now able to assess the impact of each particular knockdown on primary piRNA biogenesis or function. The disruption of known and novel factors involved in these pathways leads to elevated transposon levels, which can be detected by quantitative polymerase chain reaction (PCR). With this unbiased approach, we are going to explore new and maybe unexpected directions in this exciting field of RNAi research.

Pathways Driving Mammary Epithelial Development

C. Dos Santos [in collaboration with A. Smith, University of California, Los Angeles]

Mammary gland stem cells (MaSCs) are a highly dynamic population of cells responsible for the generation of the gland during puberty and its expansion during pregnancy. Understanding how MaSCs are regulated has become a particularly important area of research, given that they may be particularly susceptible targets for transformation in breast cancer. However, a major challenge in the field is the prospective identification of MaSC, with currently used cell surface markers (CD24+ and CD29high) only being capable of enriching for MaSCs. The improved identification of MaSCs would enable enormous insight into the mechanisms of self-renewal pertinent to normal mammary physiology and breast cancer.

To improve specific isolation of MaSCs, we are using a label-retention strategy to distinguish quiescent stem cells from their more proliferative progenitor cell populations. Our approach uses a transgenic mouse strain where histone H2b is linked to green fluorescent protein (GFP) under the conditional regulation of doxycycline (from Elaine Fuchs at Rockefeller).

Our findings indicate that mammary cells that retain H2b-GFP following transient induction are enriched for self-renewal characteristics in vitro and in vivo, beyond all currently used markers for MaSC. Moreover, global expression analysis using RNA-Seq of sorted label-retaining cells has enabled identification of novel candidate surface markers specifically expressed in H2b-GFP-positive cells compared to all other mammary gland cell types.

Ultimately, the discovery of new markers that better define the MaSC compartment may provide complementary insights into mammary gland development and mammary tumorigenesis.

Variations in Gene Expression among Single Cells

S.W. Chi [in collaboration with J. Kulik, R. Taft, Jackson lab, Cold Spring Harbor Laboratory]

A tumor is composed of a heterogeneous population of cells. One type of diversity can arise as a subpopulation of cells with multilineage potential, "cancer stem cells." According to this model, mechanisms underlying tumor heterogeneity parallel fundamental principles of differentiation processes during mammalian development. These decisions are both preceded and reflected by vari-

ations in the expression patterns of individual cells, patterns that would never be revealed in aggregate expression profiles within a tissue. A second source of diversity is the potential for different cells to harbor private mutations, contributing to tumor progression and therapy resistance. This is the driving force behind "clonal evolution." Yet, virtually all studies of developing tissues and tumors are aggregate analyses of potentially different cell types. Both types of diversities can be investigated by analysis of single cells. For this purpose, we are developing a platform to map transcriptome profiles of single cells, initially studying population diversity in developing embryos as a means to validate the approach and then using the method to analyze transcriptome and mutation diversity among single cancer cells. These studies will provide new insights into mechanisms underlying tumor heterogeneity and may impact the way we use genomic information to guide cancer therapy.

We have optimized RNA-Seq methods for profiling mRNAs in a single cell (single-cell RNA-Seq) and successfully generated cDNA from a single oocyte (~470 pg of total RNA) with great reproducibility ($R^2 = 0.8$; between two individual oocytes) and coverage (~10,000 transcripts detected; reaching the number of transcripts detected by microarray in RNA derived from an aggregate collection of thousands of oocytes). We have also successfully scaled down our procedure to smaller RNA quantities (~12.5–125 pg of total RNA) within the estimated range of RNA from developing embryos up to the 32-cell stage (~15 pg). Twelve eight-cell embryos have been collected with the aid of Rob Taft and John Kulik at the Jackson Labs. Among them, two eight-cell embryos were preliminary analyzed by single-cell RNA-Seq, showing some transcript variation in individual eight-cells. We continue to optimize the protocol and complete the rest of the eight-cell embryos to further investigate the differential expression of transcripts during embryo development.

HITMAN: Methods to Trace Neuronal Connectivity

D. Bressan [in collaboration the Zador Lab, Cold Spring Harbor Laboratory]

Our research seeks to develop a novel technique to resolve the connectivity map of a complex neural network—namely, the animal brain—with an unprecedented efficiency and throughput. Until now, all the efforts directed toward the resolution of this problem, a fundamental one in neuroscience, have used an approach based on microscopy, by imaging multiple neurons in the brain and reconstructing the pattern of their processes. This strategy makes the aim of producing a complete high-resolution (microscopic) network map extremely expensive and time-consuming. In contrast, our technique will use a molecular carrier to transport a genetic "bar code" among neurons. The circuit-specific recombination of bar codes, each one specific for a single neuron, will be read with great efficiency by next-generation sequencing, and bioinformatics will be used to reconstruct the network map. This method will allow us to map at single-cell resolution hundreds of thousands of cells in a reasonable time. In the past year, we have explored several options to find the ideal molecular "carrier" to transport the bar code. We are about to start a screen to identify novel proteins capable of moving a genetic sequence across synapses. It is our hope that this work will contribute to the broader "connectome" project as part of our collaboration with the Zador lab.

Mechanisms of Transformation in Sarcomas

E. Tonin, A. Gordon [in collaboration with R. Maestro, CRO, Aviano, Italy]

Sarcomas represent a rare form of tumor (~1% of all neoplasms) arising from still-undefined mesodermal cells. Despite their rarity, their very aggressive behavior, their often early onset, and their poor response to conventional therapies (5-year disease-free survival is less than 10%) make sarcomas one of the most challenging types of tumors. Histologically, they have been subdivided into more than 50 different categories, depending on cell shape and immunophenotipic features. Unfortunately, current classification fails to predict tumor behavior or provide useful information about treatment. Thus, a novel and more efficient classification criteria—the identification of the molecular pathways involved in the transformation of mesenchymal cells and their potential therapeutic targeting—represent important goals in the field of cancer research. With these considerations, we focus on two main projects.

First, we performed microRNA (miRNA) expression profiling of different subtypes of soft tissue sarcomas using deep-sequencing approaches to identify and quantify small RNAs involved in their development and progression. In fact, data available to date clearly support the involvement of miRNA in cancer etiology and strongly suggest their possible use as valid markers of diagnosis and prognosis and, eventually, as new targets or tools for a specific therapy. An aberrant miRNA expression is correlated with specific biopathologic features, disease outcome, and response to specific therapies in different tumor types, but very little is known

about sarcomas. We collected formalin-fixed/paraffin-embedded samples (Dr Dei Tos, Treviso City Hospital, Italy) of ~150 untreated primary sarcomas of eight different subtypes and their normal counterpart. Briefly, we extracted the total RNA, isolated the small RNA, and then prepared the cDNA libraries. We submitted them for high-throughput sequencing by Illumina. Data were analyzed using Galaxy bioinformatic tools. So far, we have collected preliminary data that must be statistically improved, increasing the number of sequenced samples for each subtype. The most representative group we have is composed of leiomyosarcomas, in which, for instance, we saw a consistent overexpression of miR-144 and miR-451 and a down-regulation of miR-1 and miR-143 (differentiation of smooth muscle cells), compared to their normal counterparts.

Second, we focused on the study of one of the most common soft tissue sarcomas in adults, the myxoid liposarcoma (MLS). Approximately 90% of cases show a characteristic t(12;16)(q13;p11) translocation, which produces the *FUS-CHOP* (*FC*) oncogene. Not much is known about this transcription factor and about how it participates in the development and progression of this neoplasia. A big step forward would be to identify all its potential binding sites. For that, we decided to use the chromatin immunoprecipitation high-throughput sequencing (ChIP-Seq) technique to find DNA fragments enriched for *FC*-binding sites. Briefly, we used the ChIP technique for *FC* in MLS cell lines and in *FC*-transfected cells (we used a dedifferentiated liposarcoma cell line as negative control). We then made a DNA library of the immunoprecipitated DNA and sequenced it by using the Solexa platform. The reads we obtained were mapped to the genome using a personalized implementation of Bowtie (an ultrafast, memory-efficient short-read aligner). We then used the peak caller MACS to identify the enriched regions. From a list of *FC* target genes (HSPA9, HSPA5, BEST1, AKT3, USP30), we predicted three possible binding-site motifs. All these data are now being confirmed by RNA-Seq analysis.

miRNA Regulation of Mouse Embryonic Stem Cells

M. Kudla

miRNAs are small RNA molecules, which have recently emerged as major regulators of various cellular processes. They act by inhibiting translation or causing degradation of the protein encoding transcript. Thanks to the cross-linking immunoprecipitation (CLIP) method, it is now possible to have a direct read-out of the miRNA target sites. This makes it possible to explain the transcriptome-wide systemic effects of miRNA. We investigate the identity of mouse embryonic stem (ES) cells from the perspective of transcripts inhibited by miRNAs. The data obtained also give insight into the cellular control of gene expression on the posttranscriptional level in ES cells.

The Long and Short of Noncoding RNAs

J. Preall

Noncoding RNAs (ncRNAs) are rapidly emerging as central players in the regulation of the epigenome, and they can act by guiding X-chromosome inactivation, DNA demethylation, imprinting, or other chromatin modifications. Chromatin organization and epigenetic regulation by ncRNAs is likely to be a complex, global phenomenon. Many potential mechanisms of ncRNA action would require their stable association with chromatin as they recruit protein machinery or guide epigenetic changes.

We have been working to identify novel ncRNA species that are stably bound to chromatin with the intention to eventually examine their correlation with epigenetic marks that accompany malignant transformation. We have developed a pulse-chase method for labeling and sequencing RNAs specifically associated with chromatin that enriches for stably associated nuclear RNAs versus nascent transcripts. This method involves metabolic labeling with modified ribonuclosides followed by in vitro biotinylation and subsequent purification. We have also been working to identify RNAs bound in the vicinity of known chromatin marks and chromatin-bound protein complexes using a modified ChIP protocol. There is also much speculation regarding the physical nature of chromatin-bound RNA, and among the possibilities is that regions of RNA:DNA hybrid duplex are formed that tether the RNA to their site of action. We are currently building sequencing libraries from material precipitated by a monoclonal antibody (S9.6) that specifically interacts with the RNA:DNA hybrid duplex structure. These approaches combined will provide a unified view of the origins, localization, and structure of chromatin-bound RNAs that are hypothesized to have important roles in epigenetic control and cell fate decisions.

The Conservation of Argonaute Catalysis

S. Cheloufi

The Argonaute proteins are highly conserved throughout evolution. They associate with diverse classes of

small RNAs to mediate gene silencing in developmental programs and participate in defense responses to viruses, transposons, and cellular stress. Vertebrate genomes encode four argonaute clade proteins. In mice, AGO2 is essential for embryogenesis, hematopoiesis, and oogenesis. The developmental functions of the remaining Argonaute family members, AGO1, AGO3, and AGO4, remain elusive. We demonstrate that individual deletions of *Ago1, Ago3, and Ago4*, or deficiency of all three Argonautes have no apparent impact on normal mouse development or fertility. In contrast to its family members, we found that AGO2 is uniquely expressed in the extraembryonic lineage and is required for proper placental development. The nucleolytic activity of Argonautes is deeply conserved, despite its having no obvious role in miRNA-directed gene silencing. AGO2 is the only family member retaining this enzymatic activity. To investigate the evolutionary pressure to conserve Argonaute enzymatic activity, we engineered a mouse with catalytically inactive *Ago2* alleles. Homozygous mutants died shortly after birth with an obvious anemia. Examination of miRNAs and their potential targets revealed a loss of miR-451, a small RNA important for erythropoiesis. Althhough this miRNA is processed by Drosha, its maturation does not require Dicer. Instead, the pre-miRNA becomes loaded into AGO2 and is cleaved by the AGO2 catalytic center to generate an intermediate 3′ end, which is then further trimmed. We demonstrate that this novel AGO2-mediated miRNA biogenesis pathway can be exploited to engineer artificial silencing molecules at both the primary miRNA and the drosha product pre-miRNA, enabeling us to hijack the pathway at different levels. These findings link the conservation of Argonaute catalysis to a conserved mechanism of miRNA biogenesis that is important for vertebrate development. Future studies using the Argonaute genetic models will help us define the biological mechanisms that dictated Argonaute gene family conservation and illuminate the wonders of the diverse RNAi pathways.

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INTEGRATED APPROACHES TO CANCER GENETICS AND BIOLOGY

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             J. Bolden   C. Fellmann   Y. Liu   Y. Park   J. Simon   S. Weissmueller
             J. Cappellani   D. Grace   A. Lujambio   P. Premsrirut   M. Spector   J. White
             C. Chen   X. Guo   B. Ma   A. Rappaport   V. Sridhar   A. Yu
             A. Chicas   C. He   K. McJunkin   M. Saborowski   N. Tasdemir   Z. Zhao
             Y. Chien   T. Kitzing   O. Mert   C. Scuoppo   M. Taylor   J. Zuber
             L. Dow

Cancer arises through an evolutionary process whereby normal cells acquire mutations that erode growth controls, leading to the expansion of aberrantly proliferating cells. Such mutations activate oncogenes or inactivate tumor suppressors, each bestowing new capabilities to developing cancer cells. Still, cancer is not an inevitable consequence of oncogenic mutations; instead, cells acquiring such mutations can be eliminated or kept in check by the intrinsic tumor suppressor programs activated in damaged cells. We previously showed that genes controlling apoptosis and cellular senescence are integral components of such intrinsic programs and, surprisingly, contribute to the tumor suppressive effects of many anticancer agents. Hence, mutations that disable tumor suppressor networks can promote tumor progression and impair treatment response.

Our research is based on the premise that the path of cancer evolution dictates a tumor's subsequent response to therapy and creates unique vulnerabilities that represent therapeutic opportunities. Hence, we apply mouse models, RNA interference (RNAi), and cancer genomics in a coordinated effort to identify compo-nents of tumor suppressor gene networks and understand molecular determinants of treatment response (Fig. 1). We use conditional RNAi technology to identify tumor maintenance genes and explore the cell intrinsic and extrinsic mechanisms involved in tumor regression (Fig. 1). Our goal is to gain a more comprehensive understanding of tumor suppressor networks and identify therapeutic targets relevant to specific cancer genotypes.

Accelerating the Pace of Discovery: Mosaic Mouse Models and RNAi

E. Antinis, C. Bialucha, J. Cappellani, L. Dow, S. Ebbesen, D. Grace, L. Lintault, K. McJunkin, C. Miething, Y. Park, P. Premsrirut, A. Rappaport, M. Saborowski, C. Scuoppo, J. Simon, V. Sridhar, M. Taylor, S. Weissmueller, J. White, Z. Zhao, J. Zuber [in collaboration with G. Hannon, S.Y. Kim, M. Camiolo, C. Malone, R. Sordella, A. Mazurek, B. Stillman, Cold Spring Harbor Laboratory]

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


Figure 1. Our laboratory studies intrinsic tumor suppressive programs involving cell death and senescence. More recently, we have studied tumor maintenance programs whose inhibition leads to the death, senescence, or differentiation of cancer cells. Often, we use RNAi to mimic tumor suppressor gene loss or model drug target inhibition.

To address these issues, we developed strategies to accelerate genetic studies in the mouse. We take a "mosaic" approach, where cancer-predisposing alleles or reporters are transduced into stem and progenitor cells derived from different tissues or mouse strains; these modified cells are then transplanted orthotopically into syngeneic recipients. Because the genetic configuration of the target cells, transduced genes, and recipients is easily varied, genotypic diversity can be readily produced. To date, we have developed mosaic models of lymphoma and leukemia, as well as carcinomas of the liver, breast, bile duct, and pancreas. Armed with these models, a single investigator can study the impact of many cancer genotypes on tumor behavior and rapidly test new hypotheses based on the results. This versatility is impossible with germline approaches.

To facilitate loss-of-gene-function studies, we worked with Gregory Hannon to develop inducible short hairpin RNA (shRNA) technology as a rapid and reversible alternative to conditional gene deletions. Recently, we showed that shRNAs embedded in a natural microRNA (miRNA) structure are effectively expressed from polymerase II promoters, thereby enabling robust gene knockdown using constitutive and inducible vectors. We then developed effective strategies for suppressing proliferation and survival genes where strong selection against shRNA expression creates technical challenges (Zuber et al. 2011). We also developed a high-throughput miRNA "Sensor" approach to functionally identify potent shRNAs (Fellmann et al. 2011). Together with Dr. Hannon, we are developing prevalidated shRNA libraries.

In vivo RNAi is a powerful tool for studying gene function in lower organisms such as *Caenorhabditis elegans* and *Drosophila*. To achieve similar capabilities in the mouse, we initially produced transgenic mice harboring a tetracycline-inducible p53 shRNA and used them to establish a role for p53 loss in tumor maintenance. We next developed an efficient and scalable platform for mouse production that involves (1) producing potent shRNAs, (2) using a recombination-mediated cassette exchange strategy developed by Jaenisch (Massachusetts Institute of Technology) to efficiently target embryonic stem (ES) cells, (3) incorporating an expression cassette that links the tet-responsive shRNA to a fluorescent reporter, (4) scaling up ES cell production, and (5) producing germline transgenic mice rapidly by tetraploid embryo complementation (Premsrirut et al. 2011).

Using this platform, we are producing hundreds of targeted ES cell lines and are developing transgenic strains capable of reversibly suppressing tumor suppressors, drug targets, and essential genes (P.K. Premsrirut et al.; K. McJunkin et al.; both in press]. Together with Gregory Hannon, we are also using this platform to produce a series of ES cells capable of inducibly expressing all known miRNAs, as well as miRNA sensors. These ES cells will be made available as a public resource to facilitate the study of miRNA biology in vivo. Finally, we are also generating improved tet *trans*-activator strains that enable potent gene knockdown in most tissues. Our goal is to develop a general platform that will enable the spatial, temporal, and reversible suppression of any mammalian gene.

Cell Survival and Cell Death

C. Bialucha, Y. Chien, C. Liu, C. Miething, C. Scuoppo, J. Simon [in collaboration with G. Hannon, Cold Spring Harbor Laboratory; J. Pelletier, McGill University]

Since its inception, our laboratory has studied genes that modulate cell death and survival. In 1993, we demonstrated that activated oncogenes could induce p53 to promote apoptosis and suppress transformation. Then, upon joining CSHL, our laboratory characterized the mechanisms of "oncogene-induced apoptosis." Some highlights include the identification of ARF as a component of oncogene signaling to p53, and several cell death effectors that mediate p53's effects. We also showed how phosphoinositol-3 kinase (PI3K) signaling can attenuate apoptosis and promote tumorigenesis, in part, via deregulated translation.

Current work in this area exploits the Eμ-*myc* B-cell lymphoma model, where disruption of the ARF-p53 pathway, or overexpression of the prosurvival genes *Bcl-2* or *Akt*, cooperates to accelerate lymphomagenesis. Following up "hits" from in vivo RNAi screens (see below), we are characterizing new tumor suppressors that modulate apoptosis. For example, we showed that Rad17 acts as a haploinsufficient tumor suppressor that mediates a DNA-damage apoptotic response to replicative stress. In unpublished work, we identified two tumor suppressors involved in polyamine biology, each of whose suppression attenuates apoptosis and promotes tumorigenesis as part of a previously obscure tumor suppressor network. Understanding this network and other hits from this screen is an important component of future research. We will also identify cell survival components that are essential for tumor maintenance. Studies using inducible shRNA transgenic mice targeting eIF4E and PTEN are already under way.

Senescence and Self-Renewal

J. Bolden, A. Chicas, Y. Chien, A. Lujambio, B. Ma, O. Mert, J. Simon, Z. Zhao, J. Zuber [in collaboration with X. Wang, C. Zhang, M. Zhang, Cold Spring Harbor Laboratory; K. Shannon, S. Kogan, University of California, San Francisco]

Cellular senescence represents an intrinsic tumor suppressive program that limits proliferation. In 1997, we discovered that—in normal cells—oncogenic *ras* triggers a senescence program involving the retinoblastoma (Rb) and p53 tumor suppressor pathways. We went on to identify factors that regulate and execute senescence and demonstrated an Rb-directed repressive chromatin state that silences growth promoting genes. Using mouse models, we also demonstrated that senescence contributes to the antiproliferative effects of cancer therapy, providing the first evidence that this process limits tumor progression in vivo. We continue to study the action of Rb, p53, and chromatin-modifying activities during senescence. By performing a genome-wide analysis of gene expression, histone modifications, and Rb chromatin association, we showed that Rb acts preferentially to suppress genes involved in DNA replication, particularly as cells exit the cell cycle into senescence (Chicas et al. 2010). We believe that this function is crucial for its tumor suppressor role.

We expanded efforts to explore the biology of senescence in vivo, recently showing that induction of senescence in tumor cells can trigger their clearance by innate immune cells. These studies reveal a non-cell-autonomous program that contributes to p53 action in tumor suppression and identifies a novel form of immune surveillance involving the innate immune system. We then discovered a role for senescence in limiting liver fibrosis, a tissue-damage response that is a predisposing factor for cirrhosis and hepatocellular carcinoma. Our results revealed the first noncancer pathology for which senescence has a protective role, and they imply that it acts to limit certain wound-healing responses. More recent studies suggest that the perforin/granzyme B pathway triggered by NK cells contributes to this clearance and that the NF-κB pathway is a master regulator of the secretory phenotype. Future studies to further dissect these and other tumor–host interactions will be greatly enabled by the use of mosaic models as well as tissue-specific inducible RNAi.

Cells that evade oncogene-induced senescence become "immortalized" in a continuous state of self-renewal. Surprisingly, recent work implies p53 limits normal stem cell self-renewal and the reprogramming of induced pluripotent stem cells. Merging these concepts, we showed that p53 loss enables the aberrant self-renewal of Ras-expressing myeloid progenitor cells, thus increasing their leukemia-initiating potential (Zhao et al. 2010). Together, these observations suggest links between p53 loss, epigenetic reprogramming, and oncogenic transformation. Future work will test whether p53 inactivation creates a distinct epigenetic state that may be targeted therapeutically.

Mechanisms of Drug Resistance and Tumor Maintenance

C. Chong, C. Miething, A. Rappaport, J. Simon, M. Spector, M. Taylor, Z. Zhao, J. Zuber [in collaboration with C. Vakoc, Cold Spring Harbor Laboratory; S. Kogan, University of California, San Francisco; M. Le Beau, University of Chicago]

Most approved cancer drugs work in only a subset of cancers, yet even these initially responsive tumors typically develop resistance. Years ago, we showed that p53 inactivation could, by disabling apoptosis, produce resistance to certain cytotoxic anticancer agents. As the first decisive example of how tumor cell genotype could influence treatment outcome, this observation stimulated our interest in understanding the interrelationship between cancer development and therapy response.

We continue to identify genes that modulate treatment sensitivity, particularly in aggressive cancers. For example, we used a lymphoma model to show that translational regulators are important determinants of rapamycin sensitivity and that NF-κB loss causes chemotherapy resistance when senescence is the dominant outcome of therapy. We used AML (acute myeloid leukemia) models to study factors that influence high-dose chemotherapy in leukemia and confirmed that p53 has a major role in this response. Although some of these effects may arise from apoptotic defects, other data suggest that p53 loss facilitates the production of so-called "cancer stem cells" considered more refractory to therapy (Zhao et al. 2010). We are currently using these models in preclinical studies to test new drugs and drug combinations.

Beyond resistance mechanisms, our work is also pointing toward new therapeutic targets. Using inducible shRNA systems, we are exploring the requirement for tumor suppressor gene inactivation in established tumors. For example, we showed that INK4a/ARF reactivation in aggressive lung carcinomas leads to tumor regression associated with enhanced apoptosis (Premsrirut et al. 2011). We also showed that Apc loss is required to maintain the proliferation of T cells in acute lymphoid leu-

kemia, thus validating the Wnt pathway as a therapeutic target (Premsrirut et al. 2011). Current efforts are studying the impact of PTEN restoration in aggressive T-cell lymphomas. Future studies will compare these effects to pharmacological inhibition of the PI3K pathway.

We are also using RNAi to identify genotype-specific drug targets. For example, by characterizing how MLL (mixed lineage leukemia) fusion proteins promote self-renewal, we identified the Myb transcription factor as specifically required for the maintenance of AMLs that are refractory to conventional chemotherapy. These observations provide direct evidence that targeting aberrant self-renewal programs can eradicate cancer. With Christopher Vakoc (CSHL), we screened an shRNA library targeting "epigenetic" regulators and have identified several histone-modifying activities whose suppression causes the selective arrest or death of leukemic cells. One of these is a target of an available small-molecule inhibitor, and preliminary studies indicate that this drug is active against our AML models and certain human AML lines.

We will continue to apply our mouse modeling and RNAi technologies to identify context-dependent tumor maintenance genes, particularly in those cancers that are refractory to current therapies. In this regard, we are already producing optimized shRNA libraries targeting relevant drug targets and developing screening strategies in vivo. Beyond mosaic models, our shRNA transgenic platform provides a rapid way to examine the normal function of any gene in whole animals as well as the potential toxic effects of transient target inhibition.

Integrated Approaches to Cancer Gene Discovery

C. Bialucha, C. Chen, S. Ebbesen, C. He, T. Kitzing, Y. Liu, B. Ma, C. Miething, A. Rappaport, M. Saborowski, C. Scuoppo, J. Simon, M. Spector, M. Taylor, S. Weissmueller, Z. Zhao, J. Zuber [in collaboration with X. Wang, National Institutes of Health; A. Kraznitz, M. Wigler, J. Hicks, G. Hannon, S. Powers, Cold Spring Harbor Laboratory]

Cancer genomes are complex and can harbor cancer-promoting "driver" mutations together with "passenger" mutations that have no biological effect. To accelerate cancer gene discovery, we use mosaic mouse models to filter through candidates obtained through genomics to identify genes that actively contribute to tumorigenesis. We presume that (1) recurrent amplifications and deletions in human tumors are enriched for oncogenes and tumor suppressors, respectively, and (2) lesions that give rise to cancer in humans often do so in mice. Since 2006, candidate testing has identified the cell death inhibitor cIAP1, the hippo pathway gene Yap, the eIF4E kinase MNK, and the chromobox protein CBX7 as potent oncogenes, as well as the Rho-GAP DLC1 as a tumor suppressor, without producing a single germline transgenic or knockout strain.

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

In parallel to these screening efforts, we are performing our own genomics analysis of human and murine cancers to facilitate functional studies in mice. Such studies have already pinpointed candidate tumor suppressors in aggressive forms of AML. Validation efforts are currently under way.

Through our screening approaches, we have noted that recurrent gene amplifications and gene deletions in tumors frequently harbor multiple drivers genes. For example, in a screen for additional tumor suppressors corresponding on human chromosome 8p—a frequent site of large heterozygous deletions in human cancers—we identified several new tumor suppressors that cooperate with DLC1 suppression to promote tumorigenesis. Moreover, in a second-generation shRNA screen using the Eμ-*myc* model described above, we used shRNA pools targeting genes deleted in human B-cell lymphoma to identify ~10 tumor suppressors. Surprisingly, four tumor suppressors corresponded to genes on human chromosome 6p, two on chromosome 8p, and two linked to p53 on 17p. These observations suggest that the impact of cancer-associated deletions provide a greater selective advantage than single gene mutations and should be viewed and studied as distinct events.

Beyond identifying new activities of cancer relevance, our approach is revealing unexpected principles about the nature and organization of cancer genes. As examples, we were surprised that so many tumor suppressors are haploinsufficient, encode secreted proteins, or have pro-oncogenic activities in other contexts. We also did not expect recurrent amplifications and deletions to contain more than one relevant activity, yet our results imply this is the rule rather than the exception. We hope

that further efforts will facilitate the functional annotation of the genomic alterations occurring in human cancers and identify vulnerabilities these lesions create.

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REPRESENTATION METHYLATION SEQUENCING ANALYSIS

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

In addition to genetic mutation, such as amplification and deletion, epigenetic aberrations are frequent events that can have far-reaching affects in the phenotype of a cancer cell. A common epigenetic modification is the methylation of cytosine residues that are next to guanine residues. It has been shown that methylation present in the DNA of the transcriptional control region has been involved in the silencing of gene expression of tumor supressors in cancer. We have previously adapted ROMA (representational oligonucleotide microarray analysis) to methylation detection oligonucleotide microarray analysis (MOMA). This methodology was useful and allowed us to identify genes that were methylated in the tumor cells, but it only gave us a general idea of where in the CpG island the methylation had occurred. In addition, after performing the analysis, sequencing validation was required to determine which of the array measurements accurately identified methylated fragments. With the development of next-generation sequencing technologies, it is now possible to identify the methylation status for the vast majority of CpG islands. Our approach is to sequence MspI representations of the genome. We have developed several different approaches to generate the libraries for sequencing and have decided to prepare Illumina libraries from bisulfite-treated MspI representations.

We have presently sequenced three tumors and two cell lines. We have determined the coverage and the number of sequences required to obtain appropriate coverage of many of the CpG islands and have developed methods to carry out the analysis on the sequencing data. Mapping the reads to the genome is not trivial because the bisulfite treatment essentially changes the genome sequence to only three bases due to the conversion of most cytosines to thymine. We have used a program developed by Andrew Smith (formerly of CSHL) to map the reads to an in silico bisulfite-treated human genome. After mapping the reads, the sequences that map to CpG islands are separated from other sequences, which represents ~20% of the reads. These reads are contigged using the program Velvet to form larger regions for analysis. The data can now be analyzed to determine gene-specific methylation states.

We have thus far used these data to identify which genes are methylated in the tumor samples. More importantly, we have expression data for these samples and will use these data to determine which region of the CpG island is methylated for genes that have suppression of transcription. In addition, it will be interesting to determine the effect of methylation of island sequences within the coding region of the gene. We plan on increasing the number of samples for two reasons. We will be analyzing a new set of ovarian tumor samples from our collaborator Dr. Douglas Levine from Memorial Sloan-Kettering Cancer Center. The data from these samples will serve as an independent validation of the genes we have found to be methylated from our MOMA results (see below). These data will also give us a much more accurate view of the regions methylated and the purpose of this methylation with respect to gene expression.

Genomic Analysis of Ovarian Cancer

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

In the United States, there will be ~22,000 new cases of ovarian cancer in 2011. Of these cases, ~14,000 will succumb to the disease. To better treat these women, and improve survival, our goal is to determine the molecular changes that have occurred in the patients' tumors and be able to interpret the significance these changes have on the growth and development of the tumor. This aberrant growth is a result of chromosomal abnormalities and epigenetic variations. In addition, generally low rates of somatic nucleotide mutation in ovarian cancer as compared to other solid tumors suggest an increased significance of copy number and epigenetic aberrations. This type of regulation has been shown to effect the many tumor suppressors and oncogenes discovered in ovarian cancer. The identification of genetic and epigenetic alterations from primary tumor cells has become a common method to identify genes critical to the development and progression of

cancer. We provide a bioinformatic analysis of copy-number variation and DNA methylation covering the genetic landscape of ovarian cancer tumor cells. We individually examined the copy-number variation and DNA methylation for 42 primary serous ovarian cancer samples using our MOMA-ROMA technology and 379 tumor samples analyzed by The Cancer Genome Atlas. We have identified 346 genes with significant deletions or amplifications among the tumor samples. Utilizing associated gene expression data, we predict 156 genes with significantly altered copy number and correlated changes in expression. Among these genes, *CCNE1, POP4, UQCRB, PHF20L1,* and *C19orf2* were identified within both data sets. We were specifically interested in copy-number variation as our base genomic property in the prediction of tumor suppressors and oncogenes in the altered ovarian tumor. We therefore identified changes in DNA methylation and expression for all amplified and deleted genes. We predicted 615 potential oncogenes and tumor suppressors candidates by integrating these multiple genomic and epigenetic data types. Genes with a strong correlation for methylation-dependent expression exhibited at varying copy-number aberrations include *CDCA8, ATAD2, CDKN2A, RAB25, AURKA, BOP1,* and *EIF2C3.* Moreover, well-characterized tumor suppressors and oncogenes exhibit varying copy-number variations, methylation, and expression features within cancers. We therefore compared significant copy-number variation and methylation effects on expression for many known tumor suppressors, oncogenes, and cancer-related genes in order to identify altered gene function properties specific to primary ovarian tumors. We analyzed multiple genomic modalities from two primary ovarian tumor data sets for the identification of candidate genes. We predict genes with tumor suppressor and oncogenic properties, identifying new serous ovarian cancer candidate genes.

Resistance Mechanisms in Ovarian Cancer

N. Cutter, M. Vigliotti, K. Wrzeszczynski

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

Genomic Alterations of Phosphatases

E. Lum, F. Chaudhary, G. Fan [in collaboration with N. Tonks, Cold Spring Harbor Laboratory]

The goal of this collaborative study is to integrate gene discovery technology with experimental strategies developed in Dr. Tonks' lab for the characterization of the protein tyrosine phosphatase (PTP) family of enzymes, to investigate how tyrosine phosphorylation-dependent signaling pathways are disrupted in cancer. We have taken advantage of several different forms of genomic data to determine which PTPs are affected in cancer cells.

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

We first generated short hairpin RNAs (shRNAs) to suppress expression of the *MTSS1* gene in cell culture as-

says, which would mimic the transcriptional repression caused by promoter methylation. Because this gene was selected based only on the comparison of tumor to normal, we did not have a selection for assay development. We surmised that because *MTSS1* possessed an actin-binding domain, the protein might have a role in cytoskeletal rearrangement, cell movement, and invasion. Therefore, we performed an invasion assay with MCF10A mammary epithelial cells, which are used typically as a normal line. We observed that very few cells migrate without the addition of the attractant epidermal growth factor (EGF). Furthermore, RNA interference (RNAi)-mediated suppression of *MTSS1* enhanced migration in the pres-

ence of EGF. There is evidence in the literature that in *ERBB2*-positive breast cancer, there is loss of *MTSS1* transcription. To determine whether *ERBB2* overexpression synergizes with loss of MTSS1, we performed an invasion assay in MCF10AN cells. These cells express a chimeric *ERBB2* gene in which the activity of the cytoplasmic PTK domain of *ERBB2* is acutely regulated by a small-molecule dimerizing agent. We noted that the level of invasion is highest in the cells in which *MTSS1* was suppressed and *ERBB2* activated, demonstrating cooperation between these two gene products. Our current data indicate that these effects are mediated via changes in tyrosine phosphorylation regulated by RPTδ.

Michele Vigliotti

MAMMALIAN FUNCTIONAL GENOMICS

A. Mills   Y. Chang   S. Paul
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            D.-W. Hwang   E. Vernersson-Lindahl
            W. Li   A. Vestin

The Mills laboratory generates novel mouse models and uses them to identify the genes responsible for human diseases of interest and to elucidate the mechanism whereby the encoded proteins regulate the disease process, with the ultimate goal of applying these findings to the human disease under study. These approaches have provided significant insight into developmental syndromes, aging, and cancer. Research areas include (1) the role of CHD5 in chromatin dynamics and cancer and (2) function of the p53 homolog p63 in development, cancer, and aging.

CHD5 in Chromatin Dynamics and Cancer

CHD5: **A new tumor suppressor mapping to human** *1p36.* *CHD5* maps to *1p36*, a region of the genome that has a three-decade history as being deleted in a wide variety of human cancers. Despite the rich body of evidence indicating that one or more tumor suppressors reside in this region, the causative gene had remained elusive. By generating mice with gain and loss of the genomic region corresponding to *1p36* using chromosome engineering technology—a strategy that allows us to generate precise rearrangements in the mouse—we pinpointed a region of the genome with potent tumor suppressive activity (Bagchi et al., *Cell 128:* 459–475 [2007]; Bagchi and Mills, *Cancer Res 68:* 2551–2556 [2008]). Using a series of genetic and molecular approaches, we identified *Chd5* as the tumor suppressor gene in the region and found that its product was a master switch for a tumor suppressive network. In addition, we discovered that *CHD5* was frequently deleted in human glioma. Identification of CHD5 as a tumor suppressor had a major impact in the cancer field, as it is now known that CHD5 is mutated in human cancers of the breast, ovary, and prostate, as well as in melanoma and neuroblastoma, and that CHD5 status correlates directly with patient survival following anticancer therapy.

Chd5 modulates chromatin dynamics and cancer. Given the critical importance of CHD5 in human cancer, we focused on elucidating its mechanism of tumor suppression. From our earlier work, we know that Chd5 transcriptionally activates the *Ink4/Arf* locus, thereby facilitating expression of p16 and p19, two tumor suppressors encoded by this locus (Bagchi et al., *Cell 128:* 459–475 [2007]). Compromised Chd5 activity, either by engineered heterozygous deletion of the interval encompassing *Chd5* or by specific depletion of Chd5 using RNA interference (RNAi), cripples the tumor suppressive network, compromising p16/Rb- and p19/p53-mediated pathways, predisposing to cancer (Mills 2010). Chd5 is a predicted chromatin remodeling protein, consistent with the idea that it regulates *Ink4/Arf* by maintaining this locus in a transcriptionally competent chromatin state.

Within the past year, we discovered that Chd5's tumor suppressive capability was dependent on its ability to bind amino-terminal tails of histone 3 (H3) that are unmodified, an activity mediated by the dual plant homeodomains (PHDs) of Chd5. Modeling provided a testable explanation for this Chd5-H3 interaction, and indeed, point mutations within the PHDs effectively abolished H3 binding, abrogated the ability of Chd5 to inhibit proliferation, and led to tumorigenesis in vivo. In line with the findings from in vitro assays, global chromatin immunoprecipitation sequencing (ChIP-Seq) analyses indicate that Chd5-bound loci tended to have less H3K4Me3 in vivo. These findings provide mechanistic insight into Chd5's ability to modulate chromatin dynamics and cancer.

Chd5-deficient mice are cancer prone. Although the chromosome engineered mouse models were invaluable for functionally pinpointing the tumor suppressive interval, the region affected was 4.3 Mb and contained 52 annotated genes. To unequivocally demonstrate the role of Chd5 in the context of spontaneous tumorigenesis, we generated mice deficient for Chd5 and monitored for tumor development. *Chd5*–/–

mice were viable, yet like we had found for mice heterozygous for the 52-gene interval encompassing *Chd5, Chd5^{+/-}* mice were prone to spontaneous tumors such as carcinoma, sarcoma, and lymphoma. We are currently analyzing these models to provide a better understanding of the biological role of CHD5 in vivo and to assess how its perturbation affects chromatin dynamics that precludes to tumorigenesis. This work should provide the groundwork for developing more effective anticancer therapies in the future.

Function of the p53 Homolog p63 in Development, Aging, and Cancer

p63 is essential for development. A long-standing focus of the laboratory has been to elucidate the in vivo role of p63—a p53-related protein that we discovered to be a new member of the p53 family. With the advent of the p53 family of proteins, the leading question in the cancer field was whether p63 (and its sibling p73) had tumor suppressive capabilities similar to p53. Using genomic technologies that we were just in the process of developing, we generated p63-deficient mouse models and discovered that p63 was essential for development of the limbs and stratified epithelia (Mills et al., *Nature 398:* 708–713 [1995]). This demonstrated that even though p63 had significant homology with p53, it had distinct biological roles in vivo. These mouse models provided a clue that revealed that p63 mutations were responsible for seven different human developmental disease syndromes. Because some of these syndrome-related p63 mutations correlated precisely with p53 hotspot mutations found in human cancer, we used a gene-targeted knockin to generate mouse models carrying these missense mutations, allowing us to recapitulate the human disease as well as to establish a link between this syndrome and cellular senescence.

p63 modulates aging and cancer. During our studies aimed at determining whether p63 had the same tumor suppressive capabilities as p53, we uncovered an unexpected link between p63, cellular senescence, and aging (Keyes et al., *Genes Dev 19:* 1986–1999 [2005]). The conditional mouse models we established were the first to implicate p63 as a modulator of cellular senescence, as well as the first to uncover a link between cellular senescence and organismal aging in vivo. Whereas p53 was known as an inducer of senescence, we found that p63 *deficiency* triggered this process. Our realization that

p63 compromised mice were not prone to spontaneous or chemically induced tumors suggested that the ability of p63 loss to trigger senescence provided a robust tumor protective mechanism that could be exploited therapeutically. Indeed, we recently reported that TAp63 isoforms are potent tumor suppressors that induce senescence and shut down growth of p53-deficient/Ras-driven tumors in vivo (Guo et al., *Nat Cell Biol 11:* 1451–1457 [2009]). Using chromosome engineering, we established conditional mouse models in which we could specifically ablate TAp63 isoforms while keeping ΔNp63 isoforms intact, allowing us to demonstrate that TAp63 isoforms prevent sarcoma development in vivo. In contrast to this tumor suppressive role of TAp63 in mesenchymal cells, we discovered that in the context of epithelial cells, ΔNp63α bypasses senescence, thereby promoting stem-like proliferation and carcinoma development in vivo (Keyes et al. 2011). We found that ΔNp63α maintains the keratin-15-positive stem cell population, promoting senescence bypass and carcinogenesis by inducing expression of the SNF2-like chromatin remodeler Lsh. These findings provide an explanation for ΔNp63α overexpression being such a frequent and highly penetrant event in human carcinoma. In addition, this work suggests that transformation of the keratin-15-expressing stem population lies at the heart of carcinoma—the most prevalent type of human cancer. Thus, our work identifies p63 as a key modulator of cellular senescence, with TAp63 isoforms being tumor suppressors that prevent sarcoma, and ΔNp63α being an oncogene that drives carcinoma development. This not only highlights the distinct tissue-specific roles of p63 isoforms; our work provides an explanation for the "tumor suppressive versus oncogenic" debate that has surrounded this p53 family member since its discovery. The double-edged tumor suppressive/oncogenic picture that has emerged for p63 is now being extended to p53 itself, as the tissue-specific roles of the multiple p53 isoforms in cancer are just beginning to be appreciated.

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CANCER GENES

S. Powers   M. Chanrion   J. Li   M. Rajaram   M. Su
             C. Eifert   J. Marchica   K. Revill   P. Tello
             M. Gallo   A. Mofunanya   E. Sawey
             R. Gerdes   H. Qi   S. Sayers

Our laboratory studies cancer in order to find out more about its basic causes but also to contribute directly to its treatment. Our primary focus has been the identification and functional validation of genetically altered driver genes (oncogenes) that together with genetically altered tumor suppressor genes are the cause of the cancer. Our approach is always to study the process from as wide a perspective as possible, which means that we consider every gene in the genome.

Identification of Driver Genes and Therapeutic Targets in Hepatocellular Carcinoma

E. Sawey, M. Chanrion, J. Li, A. Mofunanya [in collaboration with S. Lowe, Cold Spring Harbor Laboratory; R. Finn, University of California, Los Angeles; D. Chiang, University of North Carolina; D. French, Genentech]

Hepatocellular carcinoma (HCC) afflicts more than 560,000 people worldwide each year and has one of the worst 1-year survival rates of any cancer type. Currently, there are no molecular therapies that target specific mutations or other genetic alterations in HCC. By performing a forward-genetic screen guided by genomic analysis of human HCC, we identified 18 tumor-promoting genes, including CCND1 and its neighbor on 11q13.3, FGF19. Although it is widely assumed that CCND1 is the main driving oncogene of this common amplicon (15% frequency in HCC), both forward-transformation assays and RNA interference (RNAi)-mediated inhibition in human HCC cells established that FGF19 is an equally important driver gene in HCC. Furthermore, clonal growth and tumorigenicity of HCC cells harboring the 11q13.3 amplicon were selectively inhibited by RNAi-mediated knockdown of CCND1 or FGF19, as well as by an anti-FGF19 antibody. These results show that 11q13.3 amplification could be an effective biomarker for patients most likely to respond to anti-FGF19 therapy. Our study underscores the potential for clinical translation of results obtained from genetic screens guided by cancer genome analysis.

This year, we completed a pilot program to find mutations in the exome of primary HHCs, with the primary goal of finding recurrent oncogenes. On the basis of our analysis in collaboration with Derek Chiang at the University of North Carolina, *CTNNB1* is the most commonly mutated oncogene in HCC, and the rest appear to be low-frequency events, similar to what has been found in other epithelial cancers.

Of course, the best targets for treating HCC do not necessarily have to be the driver oncogenes. In principle, other alterations that occur during HCC progression could cause certain tumor dependencies that would not be found in normal liver cells. To find these, A. Mofunanya has been screening human HCC cells for tumor dependencies using genome-wide RNAi screens under conditions of hypoxia to more closely mimic the physiological state of tumor cells growing in vivo.

Identification of Driver Genes and Therapeutic Targets in Ovarian Carcinoma

E. Sawey, M. Gallo, R. Gerdes, J. Marchica, J. Li [in collaboration with A. Krasnitz, S. Lowe, Cold Spring Harbor Laboratory; K. Cho, University of Michigan; S. Orsulic, Cedars-Sinai; NCI Cancer Target Discovery and Development Network (CTD2)]

A group of scientists here at CSHL received funding from the National Cancer Institute (NCI) for a pilot project in translating genomic information emanating from cancer genome projects such as TCGA into therapeutic targets. The strategy of the CSHL CTD2 group for discovering and developing targets incorporates computational analysis of cancer genomes with oncogenomic screening and mouse modeling. This year, we have analyzed more than 500 ovarian cancer (high-grade serous) genomes and found an amazing degree of DNA copy-number instability. Through an innovative approach to make sense of this chaos, Alex Krasnitz found

the most consistently recurring alterations, and based on this analysis, we screened more than 100 of the affected genes in a transplantable mouse model for ovarian cancer and discovered that 24 of these can promote tumorigenicity. Although this is just the beginning, Shirley Guo in Scott Lowe's group has begun application of the "speedy" mouse model to determine the suitability of a subset of these oncogenes as therapeutic targets.

Studies of the DNA Methylome of HCC

K. Revill [in collaboration with R. Finn, University of California, Los Angeles; T. Wang, Massachusetts Institute of Technology]

Recent advances in whole-genome methylation profiling, specifically the Illumina Infinium platform, have allowed us to investigate the methylation status of a cohort of more than 50 HCC and normal liver samples for which we also investigated the expression and gene copy number (CGH) status. We have been able to identify two very distinct groups of HCCs based on their methylation status. In each class of tumors, there are both hypo- and hypermethylated genes relative to normal liver. Several of these tumor-specific alterations have been validated by pyrosequencing. In one class of tumors, many genes that are normally repressed by polycomb group proteins in stem cells are hypermethylated, consistent with the tumor cell of origin being a progenitor stem cell. The other class of tumors does not show this association and likely originate from a different type of normal cell. Intriguingly, these two classes also correspond to distinct groups based on both DNA copy-number analysis and expression analysis. Combining these three independent methods of global analysis—transcriptome profiling, epigenome analysis, and aCGH data—has provided a robust new classification scheme for HCC.

Identification of Sensitizers to PI3K Inhibitors

C. Eifert [in collaboration with R. Wooster, GlaxoSmithKline]

We previously reported on using Greg Hannon's RNAi library to screen for sensitizers to a polo-like kinase inhibitor and through that discovered that retinoic acid sensitizes lung cancer cells to this mode of inhibition. This year, we have looked at genes that if inhibited, together with partial inhibition of phosphoinositol-3-kinase (PI3K), could enhance killing of cancer cells. Thus far, we have validated another enzyme upstream of the PI3-biosynthetic pathway, as well as a relatively understudied glucose transporter.

Identification of Oncogenic Determinants in the Tumor Cell of Origin

M. Chanrion, E. Sawey

The notion that the phenotype of individual human tumors results from its own specific set of accumulated mutated oncogenes and tumor suppressor genes forms the major framework behind many studies into the underlying causes of specific cancer phenotypes. In contrast, it is rarely addressed whether the differentiated state of the normal cell of origin has a role in the phenotypic behavior of tumor cells following their acquisition of mutated cancer genes, despite examples where it clearly has profound effects. In fact, certain molecular subclasses of HCCs are likely to reflect different cells of origin, but the genes that underlie their different malignant properties are poorly understood. We developed an approach to find oncogenic determinants within tumor cells of origin that draws on pathway and functional analysis of different cells of origin in a mouse model integrated with parallel analysis of human HCC. Using this approach, we identified higher levels of RXRα in differentiated mouse hepatocytes and its associated subclass of human HCC as a potential oncogenic determinant. This was confirmed by demonstrating that RNAi-mediated silencing of RXRα in hepatocytes confers immature hepatoblast-like properties to their susceptibility to malignant transformation, consistent with its role in coordinating nuclear receptors involved in growth and differentiation. We also found that more differentiated HCC cells expressing higher levels of RXRα are the only type that can be inhibited by retinoids, which were previously tried on HCC patients indiscriminately. These results demonstrate the potential of this unbiased genomic approach to discover the genes underlying differential malignant properties of distinct tumor cells of origin and furthermore that such genes can be critical determinants of treatment response.

Identification and Functional Analysis of Genes Involved in Breast Cancer–Stromal Fibroblast Interactions

M. Rajaram [in collaboration with M. Egeblad, Cold Spring Harbor Laboratory]

Although some molecules that mediate the protumorigenic interaction of stromal fibroblasts and cancer cells

have been identified, there is still uncertainty about the nature and scope of all relevant interactions. To address this issue, we developed a model for breast cancer—stromal fibroblast interaction using coculture and coxenografting of human breast cancer cell lines with fibroblasts that stimulate tumorigenic growth (productive fibroblasts) or other fibroblasts that do not (nonproductive fibroblasts). By transcriptome profiling and pathway analysis, we found that the signaling pathways activated specifically in productive fibroblasts when cocultured with tumor cells overlapped significantly with pathways activated in the stroma of human breast cancer samples relative to stroma from normal breast tissue. Pathways that were up-regulated in both our model system and in human breast cancer stroma included extracellular matrix interactions, cytokine–cytokine receptor interactions, and integrin signaling. Of note was that not only were a large number of ligands and receptors up-regulated in productive fibroblasts, but their reciprocal ligands/receptors were also up-regulated in the cocultured breast cancer cells. Because this analysis pinpointed a large number of potentially important up-regulated genes, we used short hairpin RNA (shRNA) knockdown to test for their functional relevance in promoting tumor–stromal cooperativity in vivo. For example, the ability of productive fibroblasts containing stable shRNAs toward CCL2 to cooperate with breast cancer

cell lines in a coxenograft model was significantly diminished. Furthermore, a similar effect could be achieved by silencing one of its receptors—CCR1 on the breast cancer cells. Our results indicate that a productive interaction may be mediated by several different genes belonging to diverse pathways acting together to promote tumorigenesis.

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In Press

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A. Mofunanya

GENOMIC ANALYSIS OF CANCER AND HUMAN GENETIC DISORDERS

M. Wigler   J. Alexander   Y. Eberling   R. Kandasamy   A. Leotta   N. Navin   J. Rosenbaum
P. Andrews   D. Esposito   J. Katz   D. Levy   M. Oswald   J. Troge
T. Baslan   I. Hakker   J. Kendall   S. Marks   M. Riggs   Z. Wang
M. Bekritsky   J. Hicks   A. Krasnitz   J. McIndoo   L. Rodgers   B. Yamrom
K. Cook   J. Huang   Y.-H. Lee   J. Meth   M. Ronemus   Z. Zhu
H. Cox

Our group uses methods for comparative genome analysis to study cancer and human genetic disorders. New mutations, somatic and germline, underlie the cancers and human genetic disorders. Our main focus to date has been studying mutations that change the numbers of copies of sections of the genome, causing regions of deletion and duplication, in cancer and pediatric disorders. Our research in cancer is a collaborative effort to discover mutational patterns that predict clinical outcome. Additionally, we have recently developed methods for the analysis of the genomes of single cells, which has led to new insights into tumor evolution and metastasis. This work has application to the early detection of cancer and its recurrence. Our work on human genetic disorders is predicated on the hypothesis that common disorders under strong negative pressure result from new and rare variants of high penetrance. Our methodology is therefore directed to their discovery. We have made headway in the discovery of the causative mutations in autism. The same underlying theory and methodology are being applied to other disabling genetic disorders, such as congenital heart disease and pediatric cancer. All of our programs are collaborative.

Cancer

Our work in cancer is divided into three parts. First, we do outcome research (Hicks et al., *Genome Res 16:* 1465 [2006]). We use copy-number profiling to find markers that predict which breast cancers respond to which therapies. In particular, we ask which patients will respond to drugs targeting the Her-2 receptor. We are also studying ductal carcinoma in situ (DCIS) to determine if there are reliable markers that reliably predict progression to invasive disease. This work has required finding solutions to the technological problems of extracting DNA from formalin-fixed and paraffin-

embedded tissue and forming collaborations with clinical oncologists (e.g., Larry Norton at Memorial Sloan-Kettering Cancer Center and Lindsay Harris at Yale).

Second, we pursue studies to elucidate the ontogeny of cancer based on the analysis of population substructure. We used subpopulation partitioning to demonstrate the clonal heterogeneity of some breast cancers (Navin et al. 2010), and then developed single-cell sequencing techniques to study this in greater depth (Navin et al. 2011). The single-cell analysis revealed the population substructure of two cancers. One breast cancer was what we call monogenomic, with one major advanced subpopulation, and another cancer was what we called polygenomic, with multiple advanced subpopulations. No intermediate cells were found, suggesting that successful clones emerge suddenly during tumor formation, probably marking a phase change in the tumor growth pattern, and lead to metastasis. (There is an alternate hypothesis to explain the absence of intermediate cells, namely, that the cancers evolve off site, diverge greatly from their progenitors, and then reinvade the primary site.) One fundamentally surprising finding was the presence of a major subpopulation of pseudodiploid cells, each with many genomic markers, but none that resembled each other or the advanced tumor subpopulations. We suspect, but have not yet proven, that these cells are indicators of the presence of an early diploid progenitor of the tumor that benefits from, and may benefit, the advanced subpopulations.

The applications of single-cell genomic sequencing are potentially profound. Critical to all applications is developing the method so that it is inexpensive, enabling the sequencing of hundreds or even thousands of single cells in parallel for the cost of sequencing one genome in depth. Fortunately, to be useful, single-cell sequencing does not need deep coverage, so this goal is theoretically feasible. When we achieve this goal, applications include detecting cancer cells in blood and other

body fluids for the purpose of monitoring early occurrence or recurrence, extracting more information from biopsies, even directing the placement of surgical boundaries. This is the third part.

Genetic Disorders

After our discovery that copy-number variation is common in the human gene pool (Sebat et al., *Science 305:* 525 [2004]), we studied the role of copy-number variants (CNVs) in human disease and, in particular, in the role of spontaneous (or de novo) germline CNVs. Our findings established that germline mutation is a more significant risk factor for autism spectrum disorders (ASD) than previously recognized (Sebat et al., *Science 316:* 445 [2007]), and established a new approach for the further study of the genetic basis of this and other genetic disorders. We also study the role of spontaneous mutation in congenital heart disease (a collaboration with Dorothy Warburton at Columbia University), rheumatoid arthritis (with Peter Gregersen at North Shore University Hospital), and pediatric cancers (with Ken Offit at Memorial Sloan-Kettering Cancer Center, New York).

One of the de novo events we identified in autism in our 2007 paper was a deletion on 16p. This event has now been shown by two other groups to explain perhaps as much as 1% of autism. We assisted Alea Mills of CSHL to engineer mice with the orthologous deletion on mouse chromosome 7, and she has continued to search for phenotypic consequences. We are hopeful that these mice will provide animal models suitable for understanding the underlying neuropathology of the condition and the search for palliative treatments.

Analysis of autism incidence in families, a collaboration with Kenny Ye at Albert Einstein School of Medicine, provided evidence for a unified theory of the genetic basis for the disorder (Zhao et al., *Proc Natl Acad Sci 104:* 12831 [2007]). Autism families are divided into simplex (only one affected child) and multiplex (multiply affected children). By inspecting the records from the AGRE consortium, we found that the risk to a male newborn in an established multiplex family is nearly 50%, the frequency expected of a dominant disorder. Autism incidence and sibling concurrence rates are consistent with a model in which new or recent mutations with strong penetrance explain the majority of autism in males and are consistent with a one-hit event.

We are now in the midst of a larger study of spontaneous mutation in autism, based on a population of simplex families (families with only one child on the spectrum, and at least one unaffected child) collected by the Simons Foundation. Early initial results confirm our previous findings, and we observe de novo (copy-number) mutation more frequently in children with autism than in their unaffected siblings. The statistical evidence is strong for deletion events, but much weaker for amplifications, an assessment that was not possible before because of lack of statistical power. Because our new studies are performed with higher-resolution microarrays, we also see many more examples of narrow new mutations (altering only a few genes), thus expanding our list of good candidate genes involved in the disorder.

Our analysis of autism families has revealed two additional major findings. The first is statistical evidence that inheritance of rare copy-number variation also has a role in contributing to autism risk. The second is the relative absence of inherited rare deletions affecting genes compared to inherited rare duplications affecting genes. The clear implication is that most deletions are under strong negative pressure and is consonant with the observation that de novo deletions are more strongly implicated in autism than are duplications.

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

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Jean McIndoo, Ravi Kandasamy

CANCER: SIGNAL TRANSDUCTION

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

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

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

Changes in tissue architecture are often the first signs of cancer, but very little is known about the genes, proteins, and pathways that regulate cellular shape and polarity. Senthil Muthuswamy has developed a new paradigm for thinking about this aspect of cancer biology. Using sophisticated model systems such three-dimensional cell culture platforms and transgenic mice, his team found that a protein called Scribble normally regulates proper differentiation of breast epithelial cells and coerces them into the correct organization and shape and enforces resistance to cancer. They also found that deregulation of the Scribble pathway results in the development of undifferentiated tumors in mice. Muthuswamy's team finds that Scribble is frequently mislocalized from cell membranes or not expressed in human breast cancer lesions, which suggests that understanding the pathways regulated by Scribble can identify therapies aimed at preventing precancerous lesions from becoming invasive.

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

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

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 EGF (epidermal growth factor) receptor respond dramatically to its inhibition by the drug Tarceva? This occurs in 15%–20% of patients, the great majority of whom, within 1–3 years, develop resistance. Various mutations have been implicated in about half of resistant patients. This year, Sordella and colleagues discovered a new resistance mechanism in a subpopulation of NSCLC cells that are intrinsically resistant to Tarceva. These tumor cells were observed to secrete elevated amounts of a growth factor called transforming growth factor-β (TGF-β), which in turn increases secretion of interleukin-6 (IL-6), an immune signaling molecule. Significantly, these effects were independent of the EGF receptor pathway. The team therefore hypothesizes that inflammation is one factor that can render a tumor cell resistant to treatment with Tarceva.

Nicholas Tonks and colleagues study a family of enzymes called protein tyrosine phosphatases (PTPs), which remove phosphate groups from other proteins. By changing the phosphorylation state of proteins, PTPs can profoundly affect the health of entire organisms. Tonks' group seeks to characterize fully the PTP family, understand how its activity modifies signaling pathways, and how those pathways are abrogated in serious illnesses, from cancer to Parkinson's disease. The overall goal is to identify new targets and strategies for therapeutic intervention in human disease. They have, for instance, sought to define the role of JNK stimulatory phosphatase 1 in regulating a signaling pathway critical in Parkinson's and have investigated new roles for PTPs in regulating signaling events in breast cancer, identifying three PTPs as potential novel tumor suppressors. This year, Tonks and colleagues published results of experiments suggesting one way in which cascades of intracellular signals are regulated at what they call a decision point, where cells commit to repair broken DNA strands or commit suicide following DNA damage. A protein, EYA, was found by the lab to regulate the formation of specialized microenvironments on DNA called γ-H2A.X foci, which allow the cell to summon repair enzymes to the site of broken DNA strands.

Several years ago, Lloyd Trotman discovered that the loss of a single copy of a master tumor suppressing gene called *PTEN* is sufficient to permit tumors to develop in animal models of prostate cancer. His team later found that complete loss of *PTEN* paradoxically triggers senescence, an arrested state that delays or blocks cancer development in affected cells. These findings explained why many patients only display partial loss of this tumor suppressor and established a novel mechanism of cancer initiation. His lab has recently been expanding these findings in collaboration with clinicians at Memorial Sloan-Kettering Cancer Center, with the aim of identifying patients who have developed tumors with metastasis-favoring mutations. The interdisciplinary team aims to generate mouse models that accurately reflect the core genetic changes driving human metastatic prostate cancer, with the aim of developing novel molecular assays that separate the few men who are at risk of developing lethal disease from the rest. Moreover, their mouse models can be used to test new therapies using small molecules or RNA interference technology developed at CSHL. Trotman's lab also is studying regulation of PTEN stability and nuclear transport, because many patients have tumors that aberrantly target PTEN for cytoplasmic degradation.

Linda Van Aelst's lab studies how aberrations in intracellular signaling involving enzymes called small GTPases can result in disease. They are particularly interested in Ras and Rho GTPases, which help control cellular growth, differentiation, and morphogenesis. Alterations affecting Ras and Rho functions are involved in cancer and various neurodevelopmental disorders. This year, Van Aelst's team extended their study of mutations in a Rho-linked gene called *oligophrenin-1* (*OPHN1*), part of an effort to connect the genetic abnormalities associated with mental retardation to biological processes that establish and modify the function of neuronal circuits. In addition to a role for *OPHN1* in activity-driven glutamatergic synapse development, they obtained evidence that *OPHN1*

also has a critical role in mediating mGluR-LTD, a form of long-term synaptic plasticity, in CA1 hippocampal neurons. Their findings provide novel insight not only into the mechanism and function of mGluR-dependent LTD, but also into the cellular basis by which mutations in *OPHN1* could contribute to the cognitive deficits in patients. In addition, the Van Aelst team discovered a critical role for a novel activator of Rho proteins in the genesis of cortical neurons. Defects in cortical neurogenesis have been associated with cerebral malformations and disorders of cortical organization. They found that interfering with the function of the Rho activator in neuronal progenitors in embryonic cerebral cortices results in an increase in the number of proliferating neuronal progenitors and defects in the genesis of neurons. This provides novel insight into mechanisms that coordinate the maintenance of the neural progenitor pool and neurogenesis.

INTERACTIONS BETWEEN TUMOR AND HOST IN CANCER

M. Egeblad   H.A. Askautrud   M. Qiu
T. Kees   M.G. Rasch
E. Nakasone   P. Sinha
J.-H. Park

Solid tumors are composed of the cancer cells and the stroma, a supportive framework. The stroma include the extracellular matrix (ECM), which is composed of proteoglycans, hyaluronic acid and fibrous proteins (e.g., collagen), and stromal cells. The stromal cells include mesenchymal supporting cells (e.g., fibroblasts and adipocytes), cells of the vascular system, and cells of the immune system. Interactions between epithelium and stroma are essential for normal organ development. As tumors develop and progress, they undergo dramatic morphological changes, which involve both the cancer cells and the stroma. Stromal components that have been proposed to have a more pronounced tumor-promoting function in advanced stages than in earlier stages include the immune cell infiltrate.

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

Effects of the Tumor Microenvironment on Response to Cancer Therapy

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

Development of resistance can be caused by cancer cell intrinsic factors (genetic or epigenetic changes) or by extrinsic factors such as survival factors secreted from stromal cells or impairment of drug penetration through the altered tumor ECM. Certain organs, such

as the bone marrow and the thymus, have been shown to offer protection from chemotherapy mediated by secreted factors from stromal cells (e.g., interleukin 6). However, it is not known how the evolving microenvironment of solid tumors shapes drug sensitivity.

We are using in vivo spinning-disk confocal imaging to study drug sensitivity in the context of evolving tumor microenvironments. We have treated mouse models of mammary carcinoma with doxorubicin and observed very varied drug response between individual tumors. Using imaging, we revealed that the microenvironment of different tumor stages participated in regulating the drug response. Early-stage lesions responded poorly compared to intermediate-sized, early carcinoma stage tumors. However, in vitro, sensitivity to doxorubicin was similar for cancer cells from different stages. Live imaging further showed that cell death started ~24 h after doxorubicin treatment and that stromal cells as well as cancer cells in the tumor mass were killed. Treatment also led to a new microenvironment, as dead cells recruited new myeloid-derived cells, changing the microenvironment of the responding tumors. Myeloid cell infiltration was associated with increased vascular permeability, and tissue penetration of dextran and doxorubicin was most prominent in the early carcinoma stages. When vascular permeability was increased by deleting matrix metalloproteinase (MMP) 9, an improved response to doxorubicin was observed in ErbB2-driven tumors. In conclusion, imaging intact tumors acutely after treatment with chemotherapy revealed a complex and evolving relationship among microenvironment, drug penetration, and the drug sensitivity of cancer cells.

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

Y. Lazebnik   J. Milazzo

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

During the last year, we focused our limited resources on understanding the fate of tetraploid cells, which are formed by cell fusion or cytokinesis failure. According to a long-standing model, many solid cancers are caused by chromosomal aberrations consequent to tetraploidy. Two mechanisms for these aberrations have been considered: (1) spontaneous chromosome missegregation with gradual accumulation of aberrations and (2) multipolar mitosis, which can cause multiple aberrations and produce cells with ploidy characteristic of solid cancers merely by distributing four chromosome complements among three daughter cells (Fig. 1). However, the prevailing view is that such distribution is too chaotic to result in viable progeny, a minimal requirement for carcinogenesis. We questioned this assumption by analyzing multipolar mitoses caused in normal human cells

by the ability of oncoprotein E1A to inhibit centrosome clustering. We showed that during these mitoses, chromosomes distribute not as random individual entities, as commonly presumed, but in partial or complete chromosome complements, as was proposed for bipolar mitosis. We found that the resulting progeny is viable and that multipolar mitosis can frequently produce near-diploid daughters (Fig. 1), which suggests that even some near-diploid cancers might have a tetraploid precursor. Considering the track record of E1A for uncov-
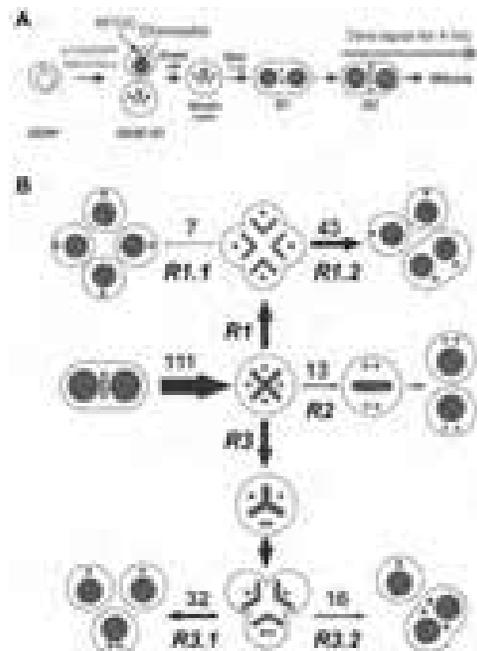
Figure 1. Multipolar mitoses caused by E1A produce diverse and viable progeny. (*A*) To visualize centrosomes and chromatin, DERP cells (DEIR transduced with p53^{R175H}) were transduced with α-tubulin–EGFP and H2A–Cherry. The resulting cell line (DERP-HT) was processed with blebbistatin to obtain binuclear cells that were recorded by confocal time-lapse microscopy as they were entering mitosis. The recordings were analyzed to determine how the cells divided (*B*). The thickness of the arrows in *B* is proportional to the number of cells that took a particular route, which were designated R1.1 to R3.2. Note that expressing α-tubulin–EGFP increased the incidence of multipolar mitoses, perhaps by interfering with the endogenous α-tubulin.

ering oncogenic pathways, and our finding that this oncogene inhibits centrosome clustering, we proposed that E1A mimics oncogenic events that cause multipolar mitosis and the consequent chromosomal aberrations in human neoplasia.

To facilitate studies on the consequences of cell fusion, in particular those to genome stability, we reported a simple, versatile, scalable, and nontoxic approach to cause cell fusion in vitro. We called this approach V-fusion, as it is based on the ability of the vesicular stomatitis virus G protein (VSV-G), a viral fusogen of broad tropism, to become rapidly and reversibly activated. We suggest that this approach will benefit a broad array of studies that investigate consequences of cell fusion or use cell fusion as an experimental tool.

During the last year, we were pleased to note that our suggestion, which we reported in 2007, that metabolism of glutamine should be revisited as a promising target for cancer therapy has been successfully implemented by a number of laboratories and pharmaceutical companies.

Finally, we published a commentary that questions the prevailing and widely accepted view of cancer as a combination of six hallmarks. We hope that this commentary will help to maintain a healthy discussion about the origin and nature of the disease that remains as incurable as it has been for decades in a majority of instances.

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

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Joseph Milazzo

EPITHELIAL CELL BIOLOGY AND CANCER

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

Pathologists use a number of criteria to diagnose and predict prognosis of premalignant breast lesions. Salient among them are changes in cell number and in cell and tissue architecture. Although we are beginning to understand a lot about the mechanisms that regulate cell proliferation, very little, if anything, is known about the mechanisms that regulate disruption of cell and tissue architecture. We believe that gaining better insights into the mechanisms that regulate changes in cell architecture during the development and progression of premalignant lesions will identify molecular targets that pathologists can use to better prognose premalignant lesions. In this process, we may also identify novel strategies to treat premalignant disease.

The epithelial cells lining the luminal space have an asymmetric distribution of membrane proteins where the membrane that is in contact with lumen, referred to as the apical surface, is rich in glycoproteins and microvilli, and the membrane that is in contact with the neighboring cell or the surrounding tissue is rich in cell–cell and cell–matrix junctions and is referred to as the basolateral surface. This characteristic organization is lost early in premalignant disease, and the tissue continues to lose its structure and organization during progression to malignancy. Surprisingly, we know little about what role these changes have during the cancer process and, more importantly, if the changes observed in premalignant lesions are harbingers of what is yet to come.

We recently demonstrated that the oncogene *ErbB2* interacts with Par6/aPKC, a protein complex involved in the regulation of asymmetric cell division, cell junction biogenesis, and epithelial morphogenesis. This interaction was required for the ability of *ErbB2* to disrupt epithelial cell polarity and transform three-dimensionally organized epithelial structures. In addition to interacting with Par6/aPKC, activation of *ErbB2* also induces disruption of Par3 from the polarity complex. We now report that expression of Par3 is down-regulated both in human breast cancers and in lung metastasis of *ErbB2* transgenic mice. Down-regulation of Par3 cooperated with the *ErbB2* oncogene to induce migration, invasion, and metastasis of transformed and tumor-derived mammary epithelial cells in culture and in vivo. Interestingly, acquisition of invasive and metastatic ability was not associated with an overt mesenchymal phenotype. However, epithelial cells lacking Par3 activated a Tiam1/Rac/GTP/IRSp53/WAVE2/Arp2/3 pathway that induced disruption of cortical actin organization, blocked E-cadherin junction maturation, and decreased cell–cell cohesion. Changes in cortical actin organization and mislocalization of Arp2/3 complex were observed in epithelial cells lacking Par3 both in culture and in primary tumors in mice and in humans. Thus, we demonstrated that loss of Par3 deregulates cortical actin dynamics and E-cadherin junction maturation to promote metastatic behavior in epithelial cells without inducing a mesenchymal phenotype.

Previous results from our lab (Zhan et al., *Cell 135:* 865 [2008]) provided evidence that overexpression of a Scribble mislocalization mutant (ScrP305L) disrupted three-dimensional morphogenesis of mammary epithelial cells, whereas overexpression of wild-type (ScrWT) Scribble did not. Therefore, we sought to investigate the signaling pathways activated in response to Scribble mislocalization in these cells. In the current study, we find that ScrP305L, but not ScrWT, specifically induces activation of Akt and phosphorylation of downstream Akt targets. To extend these observations to an in vivo setting, we generated transgenic mice expressing ScrP305L in the mammary epithelium, under the control of the mouse mammary tumor virus (MMTV) promoter. ScrP305L mice display defects in branching morphogenesis, including loss of tertiary ducts and small alveolar structures. This is followed by development of hyperplasia by 6 months of age and tumor formation after 1 year. Analysis of P305L tumors reveals a striking heterogeneity in pathology as well as marker expression, suggesting that Scribble cooperates with secondary genetic events for tumor progression. Importantly, despite their heterogeneity, ScrP305L tumors show high levels of activated Akt. Overall, our results suggest that the impaired polarization of epithelial cells noted in advanced cancers may be a cause, and not merely a consequence, of human tumorigenesis. To-

gether, these studies are likely to identify new ways for treating breast cancer.

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Sai Dipikaa Akshinthala

PROTEOMICS LABORATORY AND PROTEOMICS SHARED RESOURCE

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         C. Fu   C. Ruse
         C.M. Gauss   B. Weindorf
         A. Lelo

Identifying Guardians of Germline Genomes

This work was done in collaboration with A. Haase and G. Hannon.

The Piwi clade of Argonaute proteins and their associated small RNAs, Piwi-interacting RNA (piRNA), act in an evolutionarily conserved genome defense pathway that silences transposons in germline cells. Piwi proteins are required for germline differentiation and maintenance of germline stem cells, with mutations almost universally leading to sterility and germcell loss. Insights into piRNA biogenesis and function emanate from cloning and sequencing of piRNAs associated with different Piwi proteins, although the mechanisms of primary piRNA biogenesis and function remain largely elusive. We used affinity immunoprecipitation to identify proteins associated with *Drosophila* Piwi and three other known components of the piRNA silencing pathway: Zucchini (a potential nuclease), Armitage (an RNA helicase), and Squash (a protein of unknown function). The analysis revealed that Piwi, Armitage, and Squash associate with one another. Identification of proteins copurifying with either component revealed a common set of interactors constituting the core of this RNA-silencing machinery. The laboratory is further investigating the molecular function of candidate genes emerging from this analysis, in vitro and in vivo.

Quantitative Proteomic Analysis of the Effect of Anti-*let-7* on Protein Expression

This work was done in collaboration with C. Dos Santos, I. Ibarra, S. Obad, S. Kauppinen, and G. Hannon.

The challenge of deciphering the role of hundreds of animal RNAs, in conjunction with the large number of predicted target mRNAs, demands the development of robust technologies that can dissect the biological functions of individual microRNAs (miRNAs)

and miRNA families in vivo. An alternative approach to miRNA gene knockouts is to use chemically modified antisense oligonucleotides, termed anti-miRs, which bind to the mature miRNA in competition with cellular target mRNAs, leading to functional inhibition of the miRNA and derepression of the targets. The Hannon Laboratory has used seven to eight nucleotide fully locked nucleic acid (LNA)-modified phosphorothioate oligonucleotides (termed tiny LNAs) to target the seed region of the *let-7* family with high stability. Western blot analysis showed a concentration-dependent increase of the major HMGA2 target in anti-*let-7*-treated HeLa cells, whereas the controls showed no effect. Quantitative proteomics screens using whole-cell lysates and iTRAQ (isobaric tags for relative and absolute quantitation) labeling confirmed the increase of HMGA2 while revealing much more subtle off-target effects on other proteins. The conclusion is that tiny seed-targeting LNAs can be used to effectively inhibit the function of individual miRNAs and entire miRNA families in cultured cells. The study also validated the use of tiny LNA-based knockdown in exploring miRNA function, with important implications for the development of therapeutics targeting disease-associated miRNAs.

Mapping Posttranslational Modifications of the Splicing Factor SF2/ASF

This work was done in collaboration with Y. Liu, R.-Y. Tzeng, O. Fregoso, R. Sinha, and A. Krainer.

We used a combination of bottom-up and middle-down approaches to map a significant number of posttranslational modifications of human SF2/ASF following magnetic bead immunoprecipitation of the protein from HeLa and 293E cells. For the bottom-up approach, a triple-digest protocol was used (trypsin, elastase, and subtilisin) followed by two-dimensional MudPIT (multidimensional protein identification technology) LC-MS (liquid chromatography/mass spectrometry) analysis of

the overlapping, nonspecific digest products to increase coverage. For the middle-down approach, we used Lys-C to generate larger fragments, which were analyzed by both CID (collision-induced dissociated) and ETD (electron transfer dissociation) fragmentation. In total, we identified six sites of SY phosphorylation, six sites of KR monomethylation, and one site of R dimethylation. Eight of these sites (including four sites of phosphorylation) had not been previously reported. Subsequent work by mutagenesis and cellular assays has shown that three of the R methylations are important for controlling the partitioning of the protein between nucleus and cytoplasm. This has functional consequences for its effects on pre-mRNA splicing and nonsense-mediated decay (NMD) in the nucleus and on translation in the cytoplasm.

Novel Regulatory Modification of PTP1B

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

Hydrogen sulfide (H2S) has recently been implicated in the regulation of several biological processes, but cellular targets and possible modes of action are not well understood. Protein tyrosine phosphatases (PTPs) have been established as regulators of a wide variety of signal transduction pathways, and they use an essential reactive Cys residue, which is characterized by a low pKa and is very susceptible to oxidation. We tested whether H2S may also target the catalytic Cys residue to regulate PTP function. Using high-resolution mass spectrometry (MS) we identified a novel covalent modification (persulfation) of the active-site Cys-215 in PTP1B that occurs in response to the generation of hydrogen sulfide in vivo. This sulfhydration reaction also inactivates PTP1B, but it is subject to different mechanisms of reduction and reactivation compared to the reversibly oxidized enzyme. To understand the relevance of this modification to the control of cell signaling, we used RNA interference (RNAi) to suppress the critical enzyme in H2S production, cystathionine-γ-lyase (CSE), in 293T cells, where we observed sulfhydration of Cys-215 following ER (endoplasmic reticulum) stress only in control cells. Using a novel phosphopeptide-trapping mutant, we searched for potential substrates of PTP1B in the ER stress response and identified a tryptic phosphopeptide derived from PERK (protein-kinase-like ER kinase). The phosphorylation of Y619 in PERK has a critical role in the activation of PERK, which phosphorylates the eukaryotic translational initiation factor 2α (eIF2α), leading to translational attenuation. We have since demonstrated

that PTP1B dephosphorylates Y619 PERK and that this event is regulated by H2S production in ER stress. These data suggest the possibility that different modes of reduction and reactivation of PTPs in response to distinct gasotransmitters may introduce new levels of control over signal transduction.

Interacting Partners of MECP2

This work was done in collaboration with A. Paul and J. Huang.

MECP2 was identified as a methyl-CpG DNA-binding protein and is known to be a trancriptional modulator and regulator of chromatin structures and alternative splicing. MudPIT LC-MS analysis of MECP2 complexes obtained by magnetic bead immunoprecipitation discovered a host of new MECP2 interacting proteins. In particular, eukaryotic elongation factor 1A (EF1A1), DEAD- box protein 6 (DDX6/ P54), and synaptotagmin-binding cytoplasmic RNA-interacting protein (SYNCRIP) were identified with high significance. All three proteins have known roles in translational regulation and mRNA transport.

Because EF1A1 is integral to the translation machinery and is present in kinesin-associated mRNP granules, its interaction with MECP2 could influence the translation state of MECP2-associated mRNAs. DDX6/P54 is a well-known member of the cytoplasmic P-bodies and has been shown to repress translation and shuttle between P-body and actively translating fractions. SYNCRIP was found in kinesin-associated mRNPs as well as in dendritic mRNA transport granules. The association of MECP2 with multiple proteins that regulate translation and mRNA transport for local protein synthesis suggests a potential mechanism of how MECP2 deficiency results in altered protein levels for its target transcripts without changes in mRNA levels. Together, these results established that MECP2 not only is associated with translating mRNAs in the cytoplasm, but also imposes a regulatory function by interacting with other factors involved in mRNA metabolism and translation. The results establish a novel regulatory function of MECP2 and suggest translation regulation as a pathogenic mechanism of Rett syndrome.

Functional Analysis of the Protein Phosphatase Activity of PTEN

This work was done in collaboration with X. Zhang and N. Tonks.

The tumor suppressor phosphatase PTEN displays intrinsic activity toward both protein and phosphatidyl-

inositol phospholipid substrates in vitro. Whereas the lipid phosphatase activity of PTEN is important for its tumor suppressor function, the significance of its protein phosphatase activity is unknown and represents a gap in our understanding of the function of this important regulator of cell signaling. The Tonks laboratory has developed an in vivo assay of PTEN function, which has revealed an effect of its protein phosphatase activity on the density of neuronal spines in cultured hippocampal organotypic brain slices. In summary, the data suggest that the protein phosphatase activity of PTEN and the phosphorylation of Ser and Thr residues in its carboxy-terminal segment are of regulatory significance to the control of spine density. We are thus focused on defining these critical phosphorylation sites within PTEN and the identity of the regulatory binding proteins with which it associates.

Identification of Origin Recognition Complex Interactors

The Stillman Laboratory has identified a new mechanism by which the pre-recognition complex (pre-RC) is activated by protein kinases at the G_1-to-S phase transition that involves targeting the Cdc7-Dbf4 (DDK) protein kinase to a regulatory region of the Mcm4 protein subunit of the MCM2-7 helicase complex. The studies led to the identification of an inhibitory amino acid sequence in Mcm4 that, when deleted, bypassed the requirement for the DDK kinase. Using MCM2-7 immunoprecipitation and mass spectrometry, Cdc45 was identified as a major cell-cycle-regulated MCM-interacting protein that depended on DDK activation of the pre-RC before it could bind MCM2-7.

When Orc2 and Orc3 were depleted from human cells using RNAi, ~30% of the cells arrested with a terminal phenotype in which the mitotic chromosomes are abnormally condensed and are not attached to the mitotic spindle, and the cells have multiple centrosomes with only one centriole per centrosome. It was shown that the Orc2- or Orc3-depleted cells attempt to form a metaphase plate and then begin the metaphase-to-anaphase transition, but then the chromosomes pull off the spindles and form abnormally condensed structures. Thus, the spindle assembly checkpoint (SAC) was satisfied, but in the absence of Orc2 or Orc3, chromosomes lost spindle attachment to the kinetochores when tension was placed on the spindle to pull the centromeres apart. Subsequent biochemistry demonstrated that Orc2 and Orc3 associated with the BubR1 kineto-

chore protein that is involved in sensing tension when the kinetochores are correctly attached to bioriented spindles. Also associated with Orc2 and Orc3 were CenpE and Plk1, a protein kinase that phosphorylates BubR1, as well as the heterochromatin protein 1. The interactions between Orc2 and Orc3 at centromeres and the kinetochore proteins only occurred during mitosis and were observed during interphase; in addition, the interactions were phosphorylation dependent. We are currently performing mass spectrometry of ORC subunit immunoprecipitations to determine other proteins associated with ORC in mitosis and at centrosomes.

The Molecular Basis of Oncogene Addiction

This work was done in collaboration with M. Pineda and R. Sordella.

The Sordella laboratory is interested in the possibility that the platelet-derived growth factor receptor (PDGFR) could modify the activity of SOCS3, because it could provide a molecular mechanism explaining the decreased sensitivity to the epidermal growth factor receptor (EGFR) inhibitor in NSCLC cells expressing PDGFR. Mass spectrometry of SOCS3 was performed after in vitro phosphorylation with both PDGFR and EGFR using cold ATP. Several approaches used both the linear trap quadrupole (LTQ) and quadrupole time-of-flight (Q-TOF) mass spectrometers and in-solution and in-gel digestion of samples. Initially, several phosphotyrosines were identified within the protein, but it was suspected that they might be due to nonspecific phosphorylation. The protocol was modified by decreasing the temperature of the kinase assay and varying the concentration of the kinase, and accurate mass Q-TOF LC-MS with phospho-chip titanium dioxide enrichment was carried out. Using these approaches, the PDGFR phosphorylation site in SOCS3 was identified at amino acid 165 (Y**Y**IY), located within the gp130-binding pocket. This suggested that PDGFR may be involved in the molecular mechanism of cytokine regulation.

Quantitation of Alternative Splice Variants at the Protein Level

This work was done in collaboration with O. Fregoso, M. Jensen, A. Krainer, M. Akerman, and M. Zhang.

The Shared Resource is currently collaborating with the Krainer Laboratory to develop the use of iTRAQ quantitative proteomics to identify and quantify chang-

es in exon-junction peptides as a measure of changes in alternative splicing isoforms of abundant proteins. In multiple duplex iTRAQ experiments, protein lysates were prepared from cytoplasmic and nuclear fractions of control HeLa cells and cells overexpressing the SF2/ASF splicing factor. The lysates were digested (trypsin) and labeled with two members of the 4-plex iTRAQ reagent set before mixing. The pooled, labeled peptides were analyzed by 15-16 salt step MudPIT LC-MS or capillary LC-MS following first-dimension peptide separation by OffGel isoelectric fosusing (24 fraction; pH3-10). CID spectra are currently being matched to a splice peptide isoform database specifically created for the project.

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

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In recent years, rapid advances in our understanding of the molecular events required for tumor onset and progression have led to the development of novel cancer agents referred to as "molecular targeted therapies." Because they specifically target the product of selective cancer mutations that are required for cancer cell survival, they are thought to become invaluable therapeutic tools. Specifically in the case of lung cancer, much excitement has been generated by the finding that patients harboring oncogenic epidermal growth factor receptor (EGFR) mutations highly benefit from treatment with selective inhibitors (erlotinib and gefitinib). Erlotinib and gefitinib are members of a class of quinazolium-derived agents that inhibit the EGFR pathway by binding in a reversible fashion to the EGFR ATP pocket domain. Remarkably, retrospective studies showed a striking correlation between occurrence of certain EGFR oncogenic mutations and erlotinib/gefitinib responses. The presence of deletions in exon 19 of EGFR or EGFR L858R missense substitutions are in fact found in more than 80% of non-small-cell lung cancer (NSCLC) patients that respond to erlotinib or gefitinib treatment. Yet, as in the case of other targeted therapies, the emergence of resistance presents a major hurdle for the successful utilization of these agents. Clinical data in fact have shown that in the majority of the cases, responses to drug treatment are transient, and within a short period of time, patients who initially responded progress or relapse with resistant disease. The acquisition of an additional mutation in exon 20 of EGFR resulting in a threonine-to-methionine substitution at position 790 (T790M mutation) and/or amplification of c-MET can account for ~50% of cases of erlotinib-acquired resistance. However, the mechanisms that lead to resistance in the remaining cases are unknown.

Intrinsic and Extrinsic Mechanisms of Resistance to Targeted Therapies

To uncover new molecular mechanisms of gefitinib and erlotinib resistance in NSCLC, we have developed a cell-based model system using the broncho-alveolar cancer cell line H1650. This cell line harbors an oncogenic deletion within the EGFR (delE746-A750) and has a half-maximal inhibitory concentration (IC_{50}) to gefitinib or erlotinib treatment of ~5 μM. By culturing this cell line in the presence of a constant high concentration of erlotinib, we have been able to isolate cell lines capable of growing in the presence of up to 20 μM of the drug. Interestingly, ~13% of the erlotinib-resistant cells displayed the morphological appearance of mesenchymal cells (16 of a total of 123 colonies examined), enhanced motility, and Matrigel invasion compared to parental cells. These striking morphological features were associated at the molecular level with an increased expression of the mesenchymal protein Vimentin and with decreased expression of the epithelial marker E-cadherin, as well as with an EGFR-independent augmented secretion of transforming factor-β1 (TGF-β1) and TGF-β2.

We determined that the increased autocrine secretion of TGF-β was sufficient to activate a complex program that led to the acquisition of mesenchymal-like morphology, increased motility, invasion ability, and erlotinib resistance. In the latter case, we provided evidence that an up-regulation of interleukin-6 (IL-6) secretion by TGF-β was sufficient to unleash cells harboring mutant EGFR from their EGFR dependency, as manifested by their decreased sensitivity to erlotinib treatment.

By using a surface marker signature derived from the erlotinib-resistant cells, we also showed that cells which are mesenchymal and erlotinib-resistant were already present in NSCLC-derived cell lines as well as in early-stage treatment-naive tumors.

These data thus indicate that cell-autonomous mechanisms could generate subpopulations of cells intrinsically resistant to erlotinib treatment. Yet because both IL-6 and TGF-β are secreted factors prominently produced during the inflammatory response, the activation of the tumor microenvironment could also contribute to erlotinib resistance. By using a mouse model system in which inflammation was induced either by

topical treatment with a low concentration of 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) or with lipopolysaccharide (LPS), we were indeed able to show that the induction of inflammation was successful in stimulating IL-6 secretion and decreasing the tumor response to erlotinib treatment. Hence, our data provide compelling evidence indicating that acquired resistance to molecular targeted therapies could arise not only as a consequence of genetic and/or epigenetic heterogeneity within cancer cells, but also through the activation of the tumor microenvironment.

Because we identified cells that are intrinsically resistant to erlotinib in NSCLC prior to treatment, it was also tempting to speculate that this same mechanism of erlotinib-acquired resistance could explain the heterogeneity of primary erlotinib responses observed in patients. In fact, although the majority of patients harboring similar EGFR oncogenic mutations do respond to erlotinib treatment, overall responses can vary from 5% to 90% and remission could span from 3 months to more than 5 years. Notably, several studies have already reported increased levels of IL-6 in ~30% of NSCLC.

Why IL-6 is required for the survival of the cancer cells is yet not clear. Our data seems to indicate a role of IL-6 in protecting cells from apoptosis. This seems to be consistent with current literature. Catlett-Falcone et al. (*Immunity 10:* 105–115 [1999]) in fact demonstrated that IL-6-induced STAT3 protects myeloma cells from FAS-induced apoptosis by up-regulating the expression of BCL-X$_L$. Similarly, Haga et al. (*J Clin Invest 102:* 989 [2003]) demonstrated that constitutively active STAT3 provided protection against FAS-mediated liver injury likely through an augmented expression of the antiapoptotic proteins FLIP, BCL-2, and BCL-XL. Consistent with a role of IL-6/STAT3-mediated survival, we observed an up-regulation of Surivin, BCL-X1, and BCL-2 in the erlotinib-resistant cells.

Interestingly, the erlotinib-resistant cells characterized in this study also have an increased metastatic potential. They display mesenchymal-like features, are highly motile and invasive, and can be found with an increased representation in the bone marrow of patients compared to primary tumors. Notably, many of the genes that we see increasingly expressed in the resistant cells compared to the parental line have already been associated with a metastatic signature and/or with poor prognosis. These genes include *HOXB2, S100A4, S100A2, Tenascin C, SUSD, MCAM,* and *TCF-4.*

Notably, although the epithelial–mesenchymal transition (EMT) has been previously reported to be asso-ciated with erlotinib resistance, our data indicate that the programs that lead to mesenchymalization and drug resistance are distinct. Although we found that TGF-β is required and sufficient for EMT, invasion, and motility, as well as inducing an increased expression of IL-6, reducing the expression of the EMT master regulator SNAI only impaired EMT/motility/invasion but did not change the cells' sensitivity to erlotinib. Furthermore, treatment of cells with IL-6 increased their resistance to erlotinib but did not induce EMT. Our data are consistent with the observation that certain cell lines harboring mutant EGFR (e.g., PC9), despite being extremely sensitive to erlotinib, display mesenchymal-like features.

In conclusion, our data provide compelling evidence indicating that resistance to molecular-targeted therapies could arise not only as a consequence of genetic and/or epigenetic changes within cancer cells, but also through the activation of the tumor microenvironment. Hence, the contribution of selective and adaptive mechanisms adds a new layer to the complexity of cancer drug resistance and poses new challenges for the clinical use of molecular targeted therapies. In particular, it clearly indicates that, in the case of lung tumors driven by mutant EGFR, treatment based only on the inhibition of EGFR will not be effective and suggests the intriguing possibility that adjunctive therapies designed to either control inflammation and/or decrease the bioavailability of IL-6 may provide effective means to improve response to EGFR TKI treatment. Interestingly, clinical trials combining Cox2 inhibitors (e.g., rofecoxib and celecoxib) and gefitinib/erlotinib have already shown encouraging results.

A Novel Splice Isoform of p53 Involved in Tissue Damage Response Assists in the Bypass of Senescence and the Acquisition of the Metastatic Phenotype

Gene expression profile analysis of CD44^{high}/CD24^{low}-derived cells (H1650-M3) revealed a substantial decrease in expression of p53 compared to parental cells. Interestingly, when we validated these data by reverse transcriptase–polymerase chain reaction (RT-PCR) analysis for *p53* mRNA, we found a less-intense and slower-migrating band in the CD44^{high}/CD24^{low}-derived cells compared to parental cells. We reasoned that this band might represent an alternatively spliced transcript from the *p53* gene. Indeed, sequence analysis in-

dicated the existence in CD44^{high}/CD24^{low}-derived cells of a novel p53 alternative slice isoform that we refer to as p53Ψ. Interestingly, comparison of the *p53* gene sequence across species pointed out that the intronic sequence surrounding the alternative acceptor site in intron 6 is highly conserved.

The alternative splicing event observed in CD44^{high}/CD24^{low}-derived cells results in a frameshift placing a stop codon 19 amino acids downstream from the splice junction and the generation of a protein that is devoid of the oligomerization domain, the nuclear localization sequence (NLS) and part of the DNA-binding domain. As a consequence, p53Ψ is mainly localized in the cell cytoplasm and lacks transcriptional activity.

Yet, similarly to certain p53 mutants (i.e., R273H and R280K), p53Ψ expression leads to an increased invasion ability and decreased expression of E-cadherin. Interestingly, subcutaneous transplantation of p53-null cells (H1299) ectopically expressing either p53 wild type or p53Ψ also indicated that p53Ψ also increases the seeding capabilities of tumor cells.

Surprisingly, we noticed that the ectopic expression of wild-type p53 in the CD44^{high}/CD24^{low} cells resulted in cellular senescence. Interestingly this happens rather rapidly when compared to oncogene-induced senescence (5 days vs. 12 days in case of oncogene-induced senescence) and was exclusive to the CD44^{high}/CD24^{low} cells.

Because CD44^{high}/CD24^{low} cells express several genes that have been shown to be sufficient to drive senescence in a p53-dependent manner (e.g., IL6, IGFBP7, SMARACA2, SMARACA4, SOCS1, and SPIN1), we hypothesize that one function of this alternative splicing event is to reduce p53 activity and by doing so to prevent senescence.

One hallmark of senescent cells is the increased expression of certain secreted proteins—a phenomenon known as senescence-associated secretory phenotype, or SASP. While the overall physiological role of SASP secretion by senescent cells is still not completely understood, it has been proposed that in some cases, these proteins can induce epithelial cell proliferation and re-

sistance to apoptosis. Because the CD44^{high}/CD24^{low} cells also expressed many reported SASP components such as MMP1, MMP3, MMP10, MICA, ULBP2, PVR, and IL-6 that were found recently to be up-regulated in CD44^{high}/CD24^{low}-derived cells in activated hepatic stellate cells upon injury, we reasoned that this novel p53 alternative splicing could have a physiological significance during tissue damage/repair. We found that p53Ψ is indeed expressed in activated stellate cells during the early phase of injury/response, thus allowing these cells to linger in a presenescent state and contribute to tissue repair through the secretion of certain SASP components. Eventually, as the damage response progresses, the activated stellate cells will reexpress wild-type p53, become senescent, and be cleared in the injury/resolution phase as previously proposed.

Our experimental evidence thus indicates that p53Ψ has an important role in tissue-damage response likely by preventing premature senescence of cells involved in tissue remodeling and injury resolution.

In light of the functional and structural similarities between p53 mutants and p53Ψ, it is tempting to speculate that p53 gain-of-function mutants by functionally taking off the p53Ψ splice isoform have highjacked a highly regulated and reversible physiological response occurring during the tissue-damage response. This phenotypic mimicry may then contribute to tumorigenesis by preventing senescence, increasing cell-invasive capabilities, and enhancing the expression of CD44. Fascinatingly, tumors have been referred to as "wounds that never heal."

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes, including cell proliferation, differentiation, and survival. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects the coordinated activity of the kinases that phosphorylate it and the protein phosphatases that catalyze the dephosphorylation reaction. We study the family of protein tyrosine phosphatases (PTPs), which, like the kinases, comprise both transmembrane, receptor-linked forms and nontransmembrane, cytoplasmic species and represent a major family of signaling enzymes. Overall, the objective of the lab is to develop tools for analysis of PTP regulation and function and integrate them with state-of-the-art cell and animal models, to define critical tyrosine phosphorylation-dependent signaling events in human disease and thereby identify novel therapeutic targets. Currently, there are four broad areas of research in the lab covering functional analysis of members of the PTP family, regulation of PTP function, in particular by reversible oxidation, development of novel approaches to therapeutic intervention in PTP function, and characterization of a knockout mouse model to define the function of JSP1, a member of the PTP family that is a novel regulator of mitogen-activated protein kinase (MAPK) signaling.

During the last year, two of our graduate students, Guang Lin and Aftab Haque, successfully defended their Ph.D.s. They are currently staying in the lab while their papers are under review, but they will be moving on to pursue postdoctoral opportunities in 2011.

Identification of PTPN23 as a Novel Regulator of Cell Invasion in Mammary Epithelial Cells from a Loss-of-Function Screen of the "PTPome"

We had constructed a short hairpin RNA (shRNA) library, which contains five shRNAs specifically designed to target each of the PTPs and would allow us to interrogate the function of these signaling enzymes by RNA interference (RNAi). We conducted an RNAi-mediated loss-of-function screen to study systematically the role of the PTP superfamily of enzymes in mammary epithelial cell motility, in the absence or presence of the oncoprotein tyrosine kinase ErbB2. Although shRNAs directed against most of the PTP family members were without effect, consistent with functional specificity within this family of signaling enzymes, we identified PTPs that either promoted or inhibited motility. In particular, we have focused on three PTPs that appear to be novel tumor suppressors in breast cancer, and we are currently characterizing their mechanism of action. Suppression of PRPN23, PTPRG, and PTPRR enhanced cell motility. Furthermore, we found that suppression of PTPN23, but not PTPRG or PTPRR, induced cell invasion. Suppression of PTPN23 increased E-cadherin internalization, impaired trafficking of E-cadherin from early endosomes, induced the expression of mesenchymal proteins, and caused cell scattering. The activity of β-catenin, a regulator of cell adhesion, and SRC, a protein tyrosine kinase, were elevated when PTPN23 was suppressed. Moreover, we identified E-cadherin, β-catenin, and SRC as direct substrates of PTPN23. Inhibition of SRC with the small-molecule inhibitor SU6656 blocked the effects of PTPN23 depletion. These findings suggest that loss of PTPN23 may increase the activity of SRC and the phosphorylation status of the E-cadherin/β-catenin cell adhesion/signaling complex to promote tumor growth and metastasis in breast cancer. Our studies suggest that inhibition of SRC may offer a novel therapeutic approach to treatment of tumors in which PTPN23 is lost and, furthermore, illustrate how loss-of-function screens reveal new roles for PTPs in mammary epithelial cell biology.

Identification and Characterization of a Novel Inhibitor of PTP1B

To date, drug discovery efforts in signal transduction have emphasized the protein kinases, in particular pro-

tein tyrosine kinases. Recently, with the validation of PTP1B as a major regulator of signaling by the insulin and leptin receptors, this protein tyrosine phosphatase became a highly prized target in the pharmaceutical industry for therapeutic intervention in diabetes and obesity. In addition, the establishment of PTP1B as a critical positive regulator of signaling downstream from the HER2 oncoprotein tyrosine kinase has identified it as a candidate therapeutic target in breast cancer. Consequently, there have been major programs in industry focused on developing small-molecule inhibitors of PTP1B to address these important unmet medical needs. Nevertheless, these efforts have been frustrated by technical challenges arising from the chemical properties of the PTP-active site. The tendency of potent inhibitors to be highly charged presents problems with respect to bioavailability. Consequently, new approaches to inhibition of PTP1B, other than active-site-directed small-molecule inhibitors, are required to reinvigorate drug development efforts. We have characterized the mechanism of action of a natural product, isolated from shark liver, which we have shown to be an allosteric inhibitor of PTP1B and which is known to induce weight loss, and increase insulin sensitivity, in diet-induced and genetically obese animal models. In addition, we have observed that this natural product inhibits cell migration induced by HER2 in the MCF10A cell model of breast cancer, in a similar manner to PTP1B-directed shRNA. Through this analysis, we have identified a novel allosteric site in PTP1B outside the catalytic domain in the regulatory carboxy-terminal segment of the protein. This is important because it suggests a novel mechanism of inhibition and supports the presence of an additional binding site(s) for inhibitory small molecules within this noncatalytic portion of PTP1B. These sites would have been missed in the high-throughput screens that have been conducted to date in industry, which have used a truncated form of the enzyme comprising only the catalytic domain. Currently, we are testing this inhibitor in cell and animal models of breast cancer.

Isolation of Conformation-Sensor Recombinant Antibodies to Study Redox Regulation of PTP1B; Implications for Development of Novel Therapeutics

PTP1B is regulated by reactive oxygen species (ROS) produced in response to a wide variety of stimuli, including insulin. The reversibly oxidized form of this enzyme

is inhibited and undergoes profound conformational changes at the active site. We hypothesized that a conformation-sensor antibody that recognizes the reversibly oxidized form of PTP1B (PTP1B-OX) may stabilize the inactive state and inhibit phosphatase activity. We identified a double point mutant (C215A/S216A or CASA) in the active-site PTP motif, which adopts a stable conformation that is identical to the reversibly oxidized form (Fig. 1). We have generated a phage display antibody library consisting of single-chain variable fragments (scFvs) against this mutant PTP1B. To isolate PTP1B-OX-specific antibodies from the scFv library, we used a subtractive panning strategy. PTP1B-CASA was biotinylated at the amino terminus in *Escherichia coli* overexpressing biotin ligase and purified to homogeneity. This biotinylated CASA mutant was mixed with the library under reducing conditions in the presence of a 50-fold molar excess of wild-type PTP1B, to eliminate the scFvs that recognize the reduced conformation of the enzyme. The PTP1B-CASA-scFv-phage complex was isolated and amplified through four rounds of panning to enrich the li-
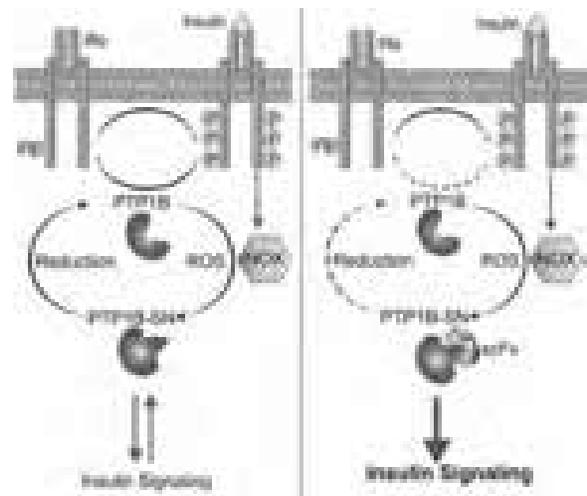
Figure 1. Expression of conformation-sensor scFv antibodies that sequester the oxidized form of PTP1B leads to enhanced insulin signaling. Insulin binding triggers autophosphorylation and activation of its receptor, which is a protein tyrosine kinase, and the phosphorylation of IRS1, its immediate substrate, which initiates the signaling response. The effects of the kinase are counterbalanced by PTPs, such as PTP1B. Activation of the insulin receptor induces the production of hydrogen peroxide (ROS) by NADPH oxidases (NOX), which leads to transient oxidation and inactivation of PTP1B. This removes the inhibitory effect of PTP1B and potentiates the signaling response. We have generated scFv antibodies that sequester PTP1B in its oxidized, inactive conformation and inhibit reduction and restoration of phosphatase activity. By trapping PTP1B in its inactive state, these antibodies enhance and sustain the signaling response to insulin.

brary with pools of antibodies selective for the mutant form of the enzyme. From the panned pools of scFvs, we have short-listed candidate antibodies in an activity-based "in-solution" screen, in which we tested the ability of individual scFvs to stabilize the oxidized, inactive form of PTP1B in enzymatic assays in vitro. These candidate scFvs displayed significant inhibition of the reactivation of PTP1B-OX by reducing agent, but they did not exert any direct inhibitory effect on activity of the reduced, wild-type enzyme. Selected scFvs were characterized further and shown to bind to the reversibly oxidized conformation of PTP1B, but not to PTP1B in its reduced active state, both in vitro and when expressed in 293T cells as intracellular antibodies or "intrabodies." Expression of the intrabody had no impact on the basal level of tyrosyl phosphorylation of either the β-subunit of the insulin receptor or its substrate IRS-1, but it enhanced and extended the time course of insulin-induced phosphorylation. Intrabody expression also caused significant enhancement of signaling downstream from the insulin receptor, as revealed by increased phosphorylation of PKB/AKT in insulin-stimulated cells. These effects on signaling were diminished when we ectopically coexpressed catalase to quench cellular H_2O_2. Our data suggest that conformation-sensor scFvs can be used as potential inhibitors of PTP1B by stabilizing the transiently inactivated form that is generated following ligand-induced production of ROS. Reversible oxidation of PTP1B results in a conformation in which the problematic chemical properties of the active site of the reduced enzyme are circumvented and new binding surfaces are presented. Therefore, if it is possible to stabilize the oxidized, inactive form of PTP1B with an appropriate therapeutic molecule that mimics the effects of these antibodies, then this strategy may provide a new approach for PTP-directed drug development that circumvents the difficulties that are encountered when targeting the highly charged PTP-active site.

Hydrogen Sulfide Signals through Sulfhydration of PTP1B

Hydrogen sulfide (H_2S), a colorless gas with the odor of rotten eggs, has been considered a toxic molecule; however, with the burgeoning interest in gasotransmitter function, it has recently been implicated in the regulation of several biological processes. H_2S is generated in a number of different mammalian tissues as a by-product of the transulfuration pathway in which dietary methionine is converted to cysteine. In order to understand the physiological function of H_2S, and its potential role in signaling, it is essential to identify its cellular targets. PTPs have been established as regulators of a wide variety of signal transduction pathways that underlie fundamentally important aspects of cell physiology. The mechanism of PTP catalysis utilizes an essential, reactive Cys residue, which is characterized by a low pKa and is susceptible to oxidation. We tested whether H_2S may also target the catalytic Cys residue to regulate PTP function. We focused on PTP1B, the founding member of this enzyme family. We observed that PTP1B was inactivated by H_2S in vitro; the reaction was reversible, as phosphatase activity was fully recovered with reducing agent. Of particular interest was the observation that H_2S-inactivated PTP1B was reactivated 60-fold faster using thioredoxin (Trx) than with dithiothreitol (DTT) and glutathione (GSH). This contrasted with oxidized PTP1B, which was reactivated to a similar (slower) rate by Trx and DTT. One potential explanation for this strong preference was provided by analysis of H_2S-inactivated PTP1B by mass spectrometry, which revealed a persulfide modification of the active-site Cys.

To understand the relevance of this modification to the control of cell signaling, we used RNAi to suppress the critical enzyme in H_2S production, cystathionine-γ-lyase (CSE), in 293T cells. As it has been demonstrated that H_2S accumulation can lead to endoplasmic reticulum (ER) stress, we tested whether PTP1B was modified during ER stress that was induced by treatment with tunicamycin. PTP1B was immunoprecipitated following ER stress from both control and CSE knockdown cells, and the modification status of the active-site Cys residue was monitored by mass spectrometry. We observed sulfhydration of the active-site Cys in control cells, which was attenuated in CSE-deficient cells. To examine further the significance of this modification, we searched for potential substrates of PTP1B in the ER stress response. The phosphorylation of Y615 in PERK (protein-kinase-like endoplasmic reticulum kinase) has a critical role in its activation; it then phosphorylates the eukaryotic translational initiation factor 2 (eIF2α), leading to translational attenuation. Our data suggest that PTP1B dephosphorylated Y615 in PERK and that this event was regulated by H_2S production in ER stress. We propose that H_2S inhibits PTP1B in the context of ER stress, thereby increasing PERK activity and in turn decreasing the rate of protein translation so as to reduce the accumulation of unfolded proteins. The susceptibility of PTPs to sulfhydration may also represent a mechanism to prevent permanent inactivation of the enzymes by oxidative

stress. These data suggest the possibility that different modes of reduction and reactivation of PTPs in response to distinct gasotransmitters may introduce new levels of control over signal transduction.

Myristoylation of the Dual-Specificity Phosphatase JSP1 Is Necessary for its Activation of JNK Signaling and Apoptosis

Activation of the c-JUN amino-terminal kinase (JNK) pathway is implicated in a number of important physiological processes, from embryonic morphogenesis to cell survival and apoptosis. JNK stimulatory phosphatase 1 (JSP1) is a member of the dual-specificity phosphatase subfamily of PTPs. In contrast to other dual-specificity phosphatases, which catalyze inactivation of mitogen-activated protein kinases, expression of JSP1 activates JNK-mediated signaling. JSP1 (and its relative DUSP15) are unique among members of the PTP family in that they contain a potential myristoylation site at the amino terminus (M<u>G</u>NGMXK). We investigated whether JSP1 was myristoylated and examined the functional consequences of myristoylation. Using mass spectrometry, we showed that wild-type JSP1, but not a JSP1 mutant in which glycine 2 was mutated to alanine (JSP1-G2A), was myristoylated in cells. Abrogation of myristoylation did not impair the intrinsic phosphatase activity of JSP1, but it changed the subcellular localization of the enzyme. Compared to wild type, the ability of nonmyristoylated JSP1 to induce JNK activation and phosphorylation of the transcription factor c-JUN was attenuated. Upon expression of wild-type JSP1, a subpopulation of cells with highest levels of the phosphatase was induced to float off the dish and undergo apoptosis; in contrast, cells expressing similar levels of JSP1-G2A remained attached. These observations highlight the importance myristoylation for JSP1 function.

Pseudophosphatases as Regulators of Cell Signaling

Throughout the PTP family are examples of pseudophosphatases, which adopt a PTP-like three-dimensional fold, but for which enzymatic activity is dispensable for their signaling function. The prototypic member, STYX, interacts with the testicular RNA-binding protein CRHSP-24 and plays an undefined role in spermatogenesis. For many transmembrane receptor-like PTPs, the intracellular segment comprises two PTP-like domains that cluster in separate clades upon sequence analysis, suggesting conserved, but distinct, functions. The membrane-proximal, D1 domains are catalytically active, whereas the membrane-distal, D2 domains are pseudophosphatases. Various roles have been postulated for these D2 domains, from redox sensors to chaperones. Pseudophosphatases are most prevalent among the myotubularins (MTMs), which regulate the phosphorylation status of phosphatidylinositol phospholipids. Of the 14 MTM genes in humans, six encode pseudophosphatases that form complexes with the active enzymes to regulate both catalytic function and subcellular location. Roles for pseudophosphatases as phospho-amino-acid-binding proteins have now also been demonstrated.

MK-STYX is a pseudophosphatase member of the dual-specificity phosphatase subfamily of the PTPs. It is catalytically inactive due to the absence of two amino acids from the signature motif that are essential for phosphatase activity. The nucleophilic Cys residue and the adjacent His, which are conserved in all active dual-specificity phosphatases, are replaced by Ser and Phe, respectively, in MK-STYX. When we generated mutations to introduce His and Cys residues into the active site of MK-STYX, we produced an active phosphatase. Ras-<u>G</u>TPase-activating protein SH3 domain-binding protein 1 (G3BP1), a regulator of RAS signaling, has been identified as a binding partner of MK-STYX. We observed that G3BP1 bound to native MK-STYX; however, binding to the mutant, catalytically active form of MK-STYX was dramatically reduced. G3BP1 is also an RNA-binding protein with endoribonuclease activity that is recruited to "stress granules" (SGs) after stress stimuli and can induce formation of SGs itself following overexpression. Stress granules are large subcellular structures that serve as sites of mRNA sorting in which untranslated mRNAs accumulate. We observed that expression of MK-STYX inhibited G3BP-induced stress granule formation; however, the catalytically active mutant MK-STYX was impaired in its ability to inhibit G3BP-induced stress granule assembly. These data reveal a novel facet of the function of a member of the PTP family, illustrating a role for MK-STYX in regulating the ability of G3BP1 to integrate changes in growth factor stimulation and environmental stress with the regulation of protein synthesis.

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Benoit Boivin

EMERGING PRINCIPLES OF TUMOR SUPPRESSION

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PTEN is a tumor suppressor that is among the most frequently lost or mutated genes of human cancer. It is unique in directly opposing the enzymatic activity of phosphoinositol 3-kinase (PI3-K) and the downstream proto-oncogene Akt kinase, which promotes cell survival and proliferation. This signaling pathway is deregulated in a majority of, for example, breast, prostate, and brain tumors. By modeling *Pten* loss in mice, we have discovered how cells in the prostate can sense the complete loss of *Pten* and respond to this insult by withdrawing into cellular senescence, which is now recognized as a cell-intrinsic human antitumoral defense mechanism. This finding defines a clear role for the p53 gene. It is essential for preventing lethal prostate cancer by mounting the senescence response after *Pten* loss, but does not initiate tumor growth when lost on its own. Moreover, the discovery of the senescence response suggests that tumors should favor retention of some PTEN. Indeed, many human cancers present with either partial loss of the *PTEN* gene or with partial functional impairment. Collectively, these findings have defined *PTEN* as a haploinsufficient tumor suppressor in several tissues and thus emphasize the need for understanding its regulation.

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

Nuclear versus Cytoplasmic Akt Activity in Cancer

M. Chen, D. Nowak, C. Pratt, M. Zeeman [in collaboration with A. Newton, University of San Diego, California; B. Carver, C. Sawyers, Memorial Sloan-Kettering Cancer Center, New York]

Through our previous studies, we have learned that not only quantitative, but also qualitative regulation of Akt kinase controls Pten mutant tumorigenesis and its progression via a senescence defeat or senescence bypass pathway. The qualitative change in active Akt kinase localization from plasma membrane to the nucleus is a direct consequence of loss of promelocytic leukemia (PML) nuclear bodies (NBs). We could show that in mice, PML achieves pAkt inactivation through its ability to recruit both Akt and its phosphatase PP2a into these bodies, resulting in specifically nuclear Akt inactivation. These findings demonstrated the importance of coordinated Akt *inactivation* and revealed that this process is efficiently achieved through the phosphatase Pp2a. Furthermore, it highlighted how deregulating cellular partitioning of Akt activity via control of phosphatases could bypass the senescence response.

Recently, a second direct phosphatase of Akt, named PHLPP, has been identified. PHLPP joins PTEN and PP2a phosphatases with the potential of regulating tumorigenesis. In contrast to the qualitative changes mentioned above, PHLPP controls the amplitude of Akt activation, similar to PTEN. By studying mice that are mutant for *Phlpp* or *Phlpp* and *Pten*, we have been able to define *Phlpp* as a tumor suppressor in prostate. It antagonizes Akt activation at the plasma membrane, and this function is crucial in preventing prostate cancer after partial loss of *Pten*. Intriguingly, combined loss of *Pten* and *Phlpp* triggers the above-mentioned p53 response, and we find that tumors need to abolish p53 to form. Thus, we find that the cooperating phosphatases direct Akt activity and localization with entirely differential outcomes with respect to p53 activation. We validate these observations in human prostate cancer progression.

Nuclear PTEN and Cancer

A. Naguib, H. Cho, M. Zeeman, C. Pratt [in collaboration with B. Carver, W. Gerald, C. Sawyers, Memorial Sloan-Kettering Cancer Center, New York]

Despite its plasma membrane function, PTEN has been consistently observed in cell nuclei, but the mechanism and relevance of this localization have remained unclear. We have recently resolved this paradox by demonstrating that contrary to polyubiquitination, nuclear PTEN

import depends on its monoubiquitination and that mutation of the main PTEN ubiquitination site abolishes import in vitro and in patients, giving rise to inheritable Cowden's Disease because of low cytoplasmic PTEN stability. But most notably, this mutant retains catalytic activity, demonstrating that PTEN nuclear import is essential for tumor suppression. These findings exemplify an elemental insight into cancer biology by demonstrating how the collaboration of a genetic lesion (the inherited mutation) with a posttranslational cellular response (enhanced degradation) cooperates in tumorigenesis. Through this analysis, we have furthermore unraveled a link between two critical means of PTEN regulation, namely, stability and nucleocytoplasmic shuttling. We are currently investigating the mediators of both processes in vitro by using RNA interference (RNAi) approaches and in vivo through gene targeting in mouse.

Regulation of PTEN Activity in Prostate Cancer Initiation and Treatment

H. Cho, M. Zeeman [in collaboration with C. Miething and P. Premsrirut, S. Lowe Lab, Cold Spring Harbor Laboratory]

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

It is important to note that such spontaneous downregulation of Pten is never observed in prostates of wild-type animals, sometimes observed in *Pten*^{+/−} prostates (30% of mice), and is always occurring in *Pten*[hy/−] animals (mice with one-fourth of normal Pten levels).

These observations strongly suggest that lower Pten levels increase the probability of its spontaneous reduction below the Akt-activating and tumor promoting threshold. Thus, they demonstrate the importance of stable Pten levels in a tissue *even after Pten has received a genetic hit*. Because this finding should form the basis of a therapeutic approach, we set out to test the therapeutic effect of Pten up-regulation in prostate. An important open question behind this approach is that restoration of PTEN might not just antagonize tumor growth, but also revert tumor growth, analogous to the concept of oncogene addiction, even after cells have suffered spontaneous cooperating lesions.

In collaboration with the lab of Dr. Lowe, we have established several mouse models that allow for RNAi-mediated reversible knockdown of *Pten* in prostate. These models now enable us to systematically test if and how established tumors respond to restoration of *Pten*. Thus, they serve as a genetic model for PI3-K-inhibitor therapy. We will also test how *Pten* restoration treatment depends on context through genetic alterations, including loss of *Phlpp*, *Pml,* and other cooperating tumor suppressors that we have identified. Through this approach, we have knocked down Pten levels in mouse prostate until tumors have formed with features that are highly similar to those of the traditional knockout models. We are now following up the effects of Pten restoration using magnetic resonance imaging (MRI) methods. Of the 250,000 American men diagnosed with prostate cancer each year, roughly half will present with partial *PTEN* loss in their lesions, and thus, we expect our results to be of great importance in establishing the genetic framework for successful treatment of prostate cancer through PTEN stabilization or PI3-K pathway inhibition.

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RAS AND RHO GTPASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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

Role for Rho-Linked Mental Retardation Protein Oligophrenin-1 at the Hippocampal CA3-CA1 Synapse

Mutations in genes encoding regulators and effectors of Rho GTPases have been found to underlie various forms of mental retardation (MR). *Oligophrenin-1* (*OPHN1*), which encodes a Rho-GTPase activating protein, was the first identified Rho-linked mental retardation gene. It was initially identified by the analysis of a balanced translocation t(X;12) observed in a female patient with mild mental retardation. Subsequent studies have revealed the presence of *OPHN1* mutations in families with mental retardation associated with cerebellar hypoplasia and lateral ventricle enlargement. All *OPHN1* mutations identified to date have been shown, or predicted, to result in *OPHN1* loss of function; however, the pathophysiological role of *OPHN1* has remained poorly understood.

We found that the OPHN1 protein is highly expressed in the brain throughout development, where it is found in neurons of all major regions, including hippocampus and cortex, and is present pre- and postsy-naptically. We have begun to unveil the function of OPHN1 both at the pre- and postsynaptic site of the hippocampal CA3-CA1 synapse. By temporally and spatially manipulating *OPHN1* gene expression, we obtained evidence that postsynaptic OPHN1 through its Rho-GAP activity has a key role in the activity-dependent maturation and plasticity of excitatory synapses by controlling their structural and functional stability. In particular, OPHN1 is recruited or stabilized in dendritic spines by spontaneous activity through the activation of *N*-methyl-D-aspartate receptors (NMDARs). In turn, OPHN1 signaling regulates activity-dependent AMPAR synaptic incorporation and stabilization, as well as maintenance of spine structure, thereby permitting synaptic maturation and plasticity. Consequently, decreased or defective OPHN1 signaling prevents glutamatergic synapse maturation and causes loss of synaptic structure, function, and plasticity. These data indicate that normal activity-driven glutamatergic synapse development is impaired by perturbation of postsynaptic OPHN1 function. More recently, we have obtained evidence that postsynaptic OPHN1 also has a critical role in mediating mGluR-dependent long-term depression (LTD), a form of plasticity relying on rapid local protein synthesis, in CA1 hippocampal neurons. This function of OPHN1 can be dissociated from its effects on activity-dependent maturation and strengthening of synapses. Together, these findings point to a multifunctional role for postsynaptic OPHN1 at CA1 synapses. Independent of its role in activity-driven glutamatergic synapse development, OPHN1 could operate to weaken synapses in response to behaviorally relevant stimuli, providing an intriguing potential explanation for some of the behavioral deficits exhibited by *OPHN1* patients.

With regard to OPHN1 presynaptic function, we found that OPHN1 is important for efficient retrieval of synaptic vesicles (SVs). The rate of endocytosis was significantly reduced in hippocampal neurons in which OPHN1 was silenced by RNA interference. In addi-

tion, presynaptic OPHN1 knockdown impaired the efficacy of synaptic transmission under high-frequency stimulation, indicating that presynaptic OPHN1 is important for maintaining synaptic efficacy during repetitive firing at hippocampal synapses. Insight into the mechanism by which OPHN1 controls SV retrieval came from our findings that OPHN1 forms a complex with endophilin A1, a protein implicated in membrane curvature generation during SV endocytosis. Importantly, *OPHN1* mutants defective in endophilin A1 binding, or with impaired Rho-GAP activity, failed to substitute for wild-type OPHN1, indicating that OPHN1's interactions with endophilin A1 and Rho are important for its function in SV endocytosis. These findings also suggest that defects in efficient SV retrieval may contribute to the pathogenesis of *OPHN1*-linked cognitive impairment.

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

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

More recently, we began to assess the role of DOCK7 in the development of cortical neurons in vivo. To this end, we implemented the in utero electroporation method that enables highly efficient introduction of vectors coexpressing proteins or short hairpin RNAs (shRNAs) of interest with a fluorescent protein

marker into ventricular zone (VZ) cells in embryonic cerebral cortices. We found that either reducing or increasing DOCK7 expression levels in progenitor cells in the embryonic cerebral cortex impairs normal migration of cortical cells, indicating that appropriate levels of DOCK7 are critical for this event. Interestingly, we also obtained evidence for a key role of DOCK7 in the genesis of neurons. In particular, we observed that DOCK7 knockdown in the VZ progenitors increases the number of proliferating S-phase cells and maintains the cells as cycling progenitors, thereby attenuating differentiation of daughter cells into neurons. On the other hand, ectopic expression of DOCK7 decreases the number of progenitors, promotes cell cycle exit, and increases the number of neurons. Thus, these findings reveal that DOCK7 function is required for cortical neurogenesis. We are currently unraveling the cellular and molecular mechanisms by which DOCK7 exerts its effects on the genesis and migration of cortical neurons, processes that have been associated with numerous neurodevelopmental disorders.

Role of Rap1 Signaling in Morphogenetic Processes

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

More recently, we have uncovered an additional role for Canoe/AF-6 as a Rap1 effector in mitotically active

epithelia, such as imaginal discs. Our experiments show that AF-6/Canoe is key to a mechanism that reconstitutes E-cadherin-based adherens junctions at the apical membrane between sister cells late in cytokinesis. A failure in this process causes cells to lose cohesion and missort during tissue morphogenesis. By conducting further genetic analysis, we found that AF-6/Canoe in this context acts on its interacting protein Echinoid, a *Drosophila* homolog of the Nectin family of IgG adhesion proteins. Echinoid expands the apical adherens junctional belt to its normal circumferential size downstream from AF-6/Canoe. Surprisingly, our functional dissection also suggests that an independent function of Echinoid acts in parallel to AF-6/Canoe in the general maintenance of adherens junctions. Together, these results suggest a more complex relationship between the Nectin and AF-6/Afadin proteins in the regulation of the mammalian adherens junctions than anticipated so far. Our observations thus provide insights into a novel molecular framework that perpetuates adherens junctional continuity in mitotically active epithelia.

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

Dok-1 (also called p62^{dok}) was initially identified as a tyrosine phosporylated 62-kD protein associated with Ras-GAP in Ph$^+$ chronic myeloid leukemia blasts and in v-Abl-transformed B cells. This protein was termed Dok (<u>d</u>ownstream <u>o</u>f <u>k</u>inases) because it was also found to be a common substrate of many receptor and cytoplasmic tyrosine kinases. Subsequently, six additional Dok family members have been identified, Dok-1 to Dok-7. Among them, Dok-1 and Dok-2 share the ability to bind to a negative regulator of Ras, Ras-GAP. We described previously that Dok-1 attenuates growth-factor-induced cell proliferation and that Dok-1 inactivation in mice causes a significant shortening of the latency of the fatal myeloproliferative disease induced by p210$^{bcr-abl}$, suggesting that it possesses tumor suppressive activity in the context of myeloid leukemia. In support of this, we found in collaboration with Dr. Pandolfi's group that mice lacking both *Dok-1* and *Dok-2* spontaneously develop a chronic myelogenous leukemia (CML)-like

myeloproliferative disease. Furthermore, more recent studies have shown that mice with combined Dok-1, Dok-2, and Dok-3 knockouts also develop lung adenocarcinoma with penetrance and latency dependent on the number of lost Dok family members.

These studies thus indicate that the Dok-1 to Dok-3 proteins possess tumor suppressive activities and that their inactivation can contribute to disease/tumor progression associated with deregulated protein kinase signaling, as, for example, in the case of p210$^{bcr-abl}$-driven CML-like disease in mice. To date, however, very little is known about the regulation of Dok proteins by oncogenic tyrosine kinases (OTKs). We have focused on Dok-1 and mechanisms of its regulation by p210$^{bcr-abl}$ and other OTKs. We found that OTKs, including p210$^{bcr-abl}$ and oncogenic forms of Src, down-regulate Dok-1 by targeting it for degradation through the ubiquitin-proteasome pathway. This process is dependent on the tyrosine kinase activity of the oncoproteins and is mediated primarily by lysine-dependent polyubiquitination of Dok-1. Importantly, restoration of Dok-1 levels strongly suppresses transformation of cells expressing OTKs, and this suppression is more pronounced in the context of a Dok-1 mutant that is largely refractory to OTK-induced degradation. Together, our findings indicate that proteasome-mediated down-regulation of Dok-1 is a key mechanism by which OTKs overcome the inhibitory effect of Dok-1 on cellular transformation and tumor progression.

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NEUROSCIENCE

CSHL neuroscientists focus on understanding how neural activity and neural circuitry underlie behavior and how disruptions in these circuits lead to neurological and neuropsychiatric disorders such as Alzheimer's disease, autism, schizophrenia, and depression. These questions are addressed in two model systems—rodents and *Drosophila*—using molecular, cellular, genetic, developmental, theoretical, physiological, and behavioral approaches. Neuroscience research at CSHL is highly collaborative and can be divided into three broad themes: sensory processing, cognition, and cognitive disorders. In addition, there is an effort to develop new anatomical methods to improve our understanding of brain circuits, connectivity, and function.

The study of decision-making provides a window on the family of brain functions that comprise 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. Anne Churchland's lab investigates the neural machinery underlying decision-making. They use carefully designed paradigms that encourage experimental subjects, both rodents and humans, to deliberate over incoming sensory evidence before making a decision. To connect this behavior to its underlying neural circuitry, they measure electrophysiological responses of cortical neurons in rodents as they perform designated tasks. The lab's current focus is on parietal cortex, which appears to be at the midpoint between sensory processing and motor planning. Churchland and colleagues also use theoretical models of varying complexity to further constrain how observed neural responses might drive behavior. This approach generates insights into sensory processing, motor planning, and complex cognitive function.

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

Grigori Enikolopov and colleagues study stem cells in the adult brain. They have generated several models to account for how stem cells give rise to progenitors and, ultimately, to neurons and are using these models to determine the targets of antidepressant therapies, identify signaling pathways that control generation of new neurons, and search for neuronal and neuroendocrine circuits involved in mood regulation. Recent experiments have suggested to the team a new model of how stem cells are regulated in the adult brain, with a focus on stem cells' decision on whether to divide—and embark on a path of differentiation—or remain quiescent. This model explains why the number of new neurons decreases with advancing age and may lead to impairments in memory and depressed mood. This year, Enikolopov and colleagues, as a part of a team of researchers, located the precise source of hematopoietic stem cell (HSC) maintenance and regulation within the bone marrow: signals emanating from another stem cell population, mesenchymal stem cells (MSCs), that create a supportive niche for the HSCs. This year, the team also identified the elusive target of deep brain stimulation (DBS), a type of precursor cell that matures into adult hippocampal neurons. Enikolopov's group is also part of a team that identified and validated the first biomarker that per-

mits neuronal progenitor cells to be tracked, noninvasively, in the brains of humans and animals. The lab is now using these discoveries to reveal how neurogenesis is related to the course of diseases such as depression, bipolar disorder, and Parkinson's.

Hiro Furukawa's lab is studying neurotransmission at the molecular level. They focus on the structure and function of NMDA (*N*-methyl-D-aspartate) receptors, ion channels that are expressed in excitatory neurons. Dysfunctional NMDA receptors cause neurological disorders and diseases including Alzheimer's disease, Parkinson's disease, schizophrenia, depression, and stroke-related ischemic injuries. NMDA receptors are very large molecules whose three-dimensional atomic structure Furukawa's group has undertaken to solve by dividing 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 NMDA receptor domains and validate structure-based functional hypotheses by a combination of biophysical techniques including electrophysiology, fluorescent analysis, isothermal titration calorimetry, and analytical centrifugation. The crystal structure of NMDA receptors will serve as a blueprint for creating and improving the design of therapeutic compounds with minimal side effects for treating neurological disorders and diseases.

To better understand neuronal circuits, Josh Huang and colleagues have developed novel means of visualizing the structure and connectivity of different cell types at high resolution in living animals and of manipulating the function of specific cell types with remarkable precision. Huang is particularly interested in circuits that use GABA (γ-amino-*n*-butyric acid), the brain's primary inhibitory neurotransmitter. The lab's work has direct implications in neurological and psychiatric illness such as autism and schizophrenia, which involves altered development and function of GABAergic circuits. This year, their work shed new light on synapse validation, which is at the heart of the process by which neural circuits self-assemble and is directly implicated in neurodevelopmental pathologies. Huang's team looked closely at neurexins, proteins that interact with neuroligins to form the "zipper" that holds synapses together. They discovered that α and β neurexins respond in different ways to neural activity—the α molecules search out compatible connection partners and the β molecules secure preliminary connections that prove to be strong. This adds to past studies in the lab on the underlying framework of molecular guides called glial cells that nudge nerve fibers to grow in the right direction and make the right contacts. Huang's lab has also made good progress in studying perturbations in the developing GABAergic system in a mouse model of Rhett's syndrome, one of the autism spectrum disorders.

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

Alexei Koulakov and colleagues are trying to figure out the mathematical rules by which the brain assembles itself, with particular focus on the formation of sensory circuits such as those in-

volved in visual perception and olfaction. The visual system of the mouse was chosen for study in part because its components, in neuroanatomical terms, are well understood. What is not known is how projections are generated that lead from the eye through the thalamus and into the visual cortex, how an individual's experience influences the configuration of the network, and what parameters for the process are set by genetic factors. Even less is known about the assembly of the neural net within the mouse olfactory system, which, in the end, enables the individual to distinguish one smell from another with astonishing specificity and to remember such distinctions over time. These are among the challenges that engage Koulakov and his team.

Dysfunction of excitatory, glutamatergic synapses in the brain is believed to have an important role in the pathogenesis of major psychiatric disorders, including schizophrenia and depression. But what are the causes? Where in the brain does this dysfunction occur? How does it result in the behavioral symptoms of illness? To address these issues, Bo Li and colleagues are studying normal synaptic plasticity and disease-related synaptic changes in brain circuits involved in schizophrenia and depression. Their long-term goal is to develop methods allowing manipulation of activity in specific brain circuits in order to change disease-related behaviors. They use a variety of methodologies, including patch-clamp recording and calcium imaging of labeled neurons, two-photon imaging of spine morphology and tagged receptors, in vivo virus injection, RNA interference (RNAi)-based gene silencing, activation of specific axon terminals using light-gated cation channels, activation or silencing of specific brain regions using transgenes, and assessment of the behavioral consequences of certain manipulations. A new project focusing initially on a gene called *ErbB4* seeks to determine the genetic causes of NMDA receptor hypofunction, a pathology believed to contribute to the etiology of schizophrenia.

Partha Mitra seeks to develop an integrative picture of brain function, incorporating theory, informatics, and experimental work. His lab is developing computational tools for integrative analysis of neurobiological data, spanning electrophysiological, neuroanatomical, and genomic data from multiple species pertaining to the brain. In the Mouse Brain Architecture Project, Mitra and colleagues are well on their way to generating the first-ever brainwide mesoscopic-scale circuit map of the entire mammalian brain. This is a first step toward mapping vertebrate brain architecture across species and in mouse models of human disease, starting with schizophrenia and autism. These maps will be cross-referenced to the Allen Brain Atlas of gene expression, a complementary landmark data set on which the Mitra lab has innovated multiple analyses. These analyses include assessing coexpression patterns and networks of addiction-related genes and determining evolutionary ages of genes with respect to their brain region of expression.

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

Jonathan Sebat's lab studies the role of genetic variation, and particularly, gene copy-number variation, in schizophrenia, autism, and other neuropsychiatric illnesses. Sebat and collaborators have discovered a rare mutation associated with dramatically increased schizophrenia risk, a gene copy-number variation in a chromosomal region, 16p11.2, already linked to autism. Previously, they had demonstrated a significantly increased rate of rare structural mutations in the genomes of people with schizophrenia as compared with healthy controls. They found, moreover, that the mu-

tations were powerful and that the genes disrupted by the mutations nearly half the time were involved in pathways known to be important in brain development. One implication is that the universe of genetic risk factors for schizophrenia consists of many different rare mutations, each one present in comparatively few individuals, or even a single one. In prior work, Sebat, in collaboration with Michael Wigler, discovered that spontaneous mutations—genetic errors in children that do not occur in either parent—are far more common in autism than previously thought.

Stephen Shea's lab studies the neural circuitry underlying social communication and decisions. He uses natural social communication behavior in mice as a model to understand circuits and processes that are evolutionarily conserved and therefore shared broadly across species, likely contributing to disorders such as autism. Previously at Duke University, Shea and colleagues examined how emotion and arousal enable mice, via their olfactory systems, to store memories of other individuals and of related social signals. The team exploited the intimate relationship between memory and emotion to effectively create memories in anesthetized mice, providing unprecedented access to neurobiological processes that typically only occur during behavior. To advance understanding of the circuit mechanisms of these memories, this year Shea's lab developed techniques to make technically challenging targeted recordings from a specific type of inhibitory neuron whose function remains mysterious. The data suggest that these cells not only participate in storing olfactory memories but do so in surprising and complex ways. Also this year, Shea's team began a series of studies of a different form of social recognition: auditory recognition of pup vocalizations by their mothers. Preliminary data show that the brains of mother mice encode the sound of a pup's voice differently, and this may relate to differences in the quality of their maternal care. Ultimately, the Shea lab aims to include recordings from awake and behaving mice and other techniques to understand the social decision process all the way from the level of a live social encounter to the movement of neurotransmitter receptors at the synapse.

What is a memory? When we learn an association, information from two different sensory streams 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 that it becomes linked to that taste? What are the underlying mechanisms—what synapses change strength or what ion channel properties change? These are the questions that drive research in Glenn Turner's laboratory. His team addresses these questions by monitoring neural activity using a combination of electrophysiological methods to examine individual neurons with very high resolution and functional imaging to track the activity of hundreds of neurons simultaneously. They have developed optical 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. By examining the effects of learning-related genes on this process, they can connect their network-level view of memory formation to the underlying molecular mechanisms governing the basic cellular and synaptic changes that drive learning.

Anthony Zador and colleagues study how brain circuitry gives rise to complex behavior. Work in the lab is focused on two main areas. First, they ask how the cortex processes sound, how that processing is modulated by attention, and how it is disrupted in neuropsychiatric disorders such as autism. Second, they are developing new methods for determining the complete connectivity of the mouse brain at single-neuron resolution. In contrast to previous methods that make use of microscopy, these methods exploit high-throughput DNA sequencing. Because the costs of DNA sequencing are plummeting so rapidly, these methods have the potential to yield the complete wiring diagram of an entire brain for mere thousands of dollars.

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 that longer resting intervals for Noonan's patients might reverse their memory deficits. This year, they identified a means of reversing memory loss in fruit flies while suppressing brain plaques similar to those implicated in Alzheimer's disease. Separately, having discovered that memory decay is an active process, regulated by the Rac protein, the team has proposed that Rac's role in erasing memory is related to its influence on downstream cytoskeleton remodeling agents.

NEURAL CIRCUITRY FOR PERCEPTUAL DECISIONS

A. Churchland   D. Raposo
              H. Zamer

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

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

Our long-term goal is to understand how the brain can make decisions that integrate inputs from our multiple senses, stored memories, and innate impulses. Our current projects, described below, are a first step toward this complete understanding of perceptual decision-making.

Measuring Behavior on a Multisensory Decision-Making Task

We present both rat and human subjects with ambiguous stimuli about which they make decisions. The stimuli consist of visual flashes, auditory tones, or both. Subjects judge the repetition rate of the stimulus. To determine whether subjects benefit from multisensory integration, we compare the speed and accuracy of the single sensory and the multisensory conditions.

Uncovering the Neural Mechanisms of Multisensory Decision-Making

We collect electrophysiological responses from single neurons in the parietal cortex. The parietal cortex is known to receive inputs from both auditory and visual sensory areas and is known to have a key role in decision-making. By comparing the electrophysiological responses of parietal neurons when the animals are engaged in single sensory versus multisensory decisions, we can explore the nature of the cortical circuitry that makes it possible to support the behavioral improvement in multisensory integration.

Connecting Behavior and Physiology Using Theoretical Models

Behavior and physiology from decision tasks about visual motion have been consistent with a class of models known as bounded accumulation. In these models, random samples of evidence are accumulated over time up

to a threshold level or bound. Although this class of model has been successful for visual motion decisions, it is not yet known whether it will generalize to multisensory decisions. Our working hypothesis is that the models will be easily extendable to multisensory decisions: Instead of accumulating random samples of evidence only across time, the models can be designed to accumulate random samples of evidence across modalities. We are in the process of testing whether this class of models can accurately predict the speed and accuracy of decisions, and whether its predictions are compatible with electrophysiological observations. Other kinds of models that are currently under consideration are models of Bayesian inference, or attractor models.

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H. Zamer, A. Churchland

GENETICS OF MEMORY IN *DROSOPHILA*

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

Understanding complex behaviors such as memory will require a multidisciplinary approach that will include discovery and manipulation of the relevant genetic/cell signaling pathways and the relevant neural circuits. Work in genetic model systems such as *Drosophila* can contribute to our understanding in several ways. First, by enabling discovery of genes and genetic pathways underlying behavior, such model systems provide entry points for dissection of cellular mechanisms that are often conserved. Second, systematic manipulation of gene function in relevant anatomical loci of the brain allows a conceptual integration of findings from cellular, neuroanatomical, and behavioral levels. Our gene discovery efforts already have identified a role in memory for highly conserved pathway(s) involved with subcellular control of translational regulation. Many of these molecules have counterparts in vertebrates that also appear to have important roles in brain function and, in some cases, may be linked to human cognitive dysfunction. Our genetic investigations also provide insight to investigate the neural circuitry relevant to memory because gene expression patterns often suggest hypotheses that can be tested spatially restricted genetic manipulations.

Neuronal Translational Regulatory Network

W. Li, L. Prazak, J. Dubnau

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

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

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

Synaptic targets of pumilio. We identified a role in memory for a number of mRNA-binding proteins (Dubnau et al., *Curr Biol 13:* 286 [2003]). These include several with known roles in mRNA localization and translational control, including *staufen* and *oskar*, which are known components of a cellular mRNA localization machinery in oocytes, and *pumilio*, which is a translational repressor protein whose vertebrate orthologs are highly conserved but largely unstudied. Although *pumilio* function has been most carefully studied in the context of embryonic patterning, several recent findings demonstrate that *pumilio* also has an important role in the nervous system, including long-term memory formation (see, e.g., Berger et al., *Alcohol Clin Exp Res 32:* 895 [2008]; Chen et al., *PLoS Comput Biol 4:* 340 [2008]; Dubnau et al., *Curr Biol 13:* 286 [2003]). In neurons, *pumilio* appears to have a role in homeostatic control of excitability via down-regulation of *para*, a voltage-gated sodium channel, and may more

generally modulate local protein synthesis in neurons via translational repression of *eIF-4E*. Aside from these, the biologically relevant targets of *pumilio* in the nervous system were largely unknown in any species.

We hypothesized that *pumilio* might have a role in regulating the local translation underlying synapse-specific modifications during memory formation. To identify relevant translational targets, we used an informatics approach (in collaboration with M. Zhang's lab at CSHL) to predict *pumilio* targets among mRNAs whose products have synaptic localization (Chen et al., *PLoS Comput Biol 4:* 340 [2008]). We then used both in vitro binding and two in vivo assays to functionally confirm the fidelity of this informatics screening method. We found that *pumilio* strongly and specifically binds to RNA sequences in the 3′UTR (untranslated region) of four of the predicted target genes, demonstrating the validity of our method. We then demonstrated that one of these predicted target sequences, in the 3′UTR of *discs large* (*dlg1*), the *Drosophila* PSD95 ortholog, can functionally substitute for a canonical Nanos response element (NRE) in vivo in a heterologous functional assay in the embryo. We also demonstrated that endogenous levels of *dlg1* (Chen et al., *PLoS Comput Biol 4:* 340 [2008]) and another neuronal target, 14-3-3, can be regulated by *pumilio* in a neuronal context, the adult mushroom bodies (MB), an anatomical site of memory storage. Our current efforts focus on several additional neuronal targets of *pumilio* as well as on putative cofactors that act in concert with *pumilio* in the early embryo.

Small-RNA-mediated gene regulation in the brain. microRNAs (miRNAs) are ~21–23-nucleotide noncoding RNA transcripts that regulate gene expression at the posttranscriptional level by binding to complementary sequences in the 3′UTRs of target mRNAs. miRNAs function as part of an RNA-induced silencing complex (RISC) that includes one of the argonaute protein family members. In *Drosophila*, miRNAs are most often associated with argonaute-1. Via a forward mutagenesis screen for defective memory, we identified mutations that mapped to an miRNA gene (Dubnau et al., *Curr Biol 13:* 286 [2003]). Using classical *Drosophila* molecular genetics, we have confirmed a causal relationship between the disrupted expression of the miRNA and the behavioral defect. We also have identified a role in memory for argonaute-2, rather than argonaute-1, and have also shown that argonaute-1 and *pumilio* interact genetically. These and other findings suggest a core translational regulatory mechanism in the brain in which common mRNA targets are regulated coordinately by *pumilio* and by a RISC complex involving small RNAs loaded onto argonaute-2.

Neural and Genetic Circuitry of Memory

M. Cressy, H. Qin, J. Dubnau

A common feature of memory and its underlying synaptic plasticity is that each can be dissected into short-lived forms involving modification or trafficking of existing proteins and long-term forms that require new gene expression. An underlying assumption of this cellular view of memory consolidation is that these different mechanisms occur within a single neuron. At the neuroanatomical level, however, different temporal stages of memory can engage distinct neural circuits, a notion that has not been conceptually integrated with the cellular view. A recent study in our lab has highlighted the significance of this integrated view. Blum et al. (*Curr Biol 19:* 1341 [2009]) has shown that *rutabaga* adenylyl cyclase function is required in a specific neuronal cell type in a learning center called the mushroom bodies. These so-called γ-lobe neurons require rutabaga function to support short-term memory (STM), but in contrast, rutabaga expression is needed in α/β-lobe neurons to support long-term memory (LTM). On the basis of this observation, Blum et al. (*Curr Biol 19:* 1341 [2009]) and Blum and Dubnau (2010) proposed a hypothesis that two parallel STM traces are formed in the MBs, in different cell types. To test this model, we have established a genetic means to generate animals that are capable of forming only the rutabaga-dependent or only the rutabaga-independent STM forms. This allows us to investigate the cellular substrates and consolidation kinetics of each of these genetically distinct memory traces.

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

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

One of the great challenges in understanding the genetic impact on human disease is that some complex disorders, such as schizophrenia, likely emerge from coinheritance of multiple common gene variants, each of which would have little clinical impact on their own. Despite their widespread relevance, mechanisms by which multi-

gene interactions modulate phenotype are ill-understood because almost all mechanistic studies of gene interaction are limited to pair-wise studies. To investigate this question, we have developed and implemented a novel approach in *Drosophila*, using the biologically important and clinically relevant cAMP pathway as a model. We used selective breeding to evolve combinations of alleles capable of suppressing the learning defect associated with mutations in the *rutabaga* adenylyl cyclase gene. Unlike a classical suppressor screen, our use of experimental evolution allows us to explore the potential impact of higher-order gene interactions. And unlike a classical selective breeding experiment, we constrained the genetic variability to a set of 23 known loci, providing access to

the underlying causal alleles. Using independent genetic experiments, we exhaustively tested the effects of each of the identified loci as well as of all diallele combinations. Our results indicate that numerous genotypic solutions are present and that typical solutions involve combinations of between three and six loci.

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Michael Cressy

STEM CELLS, SIGNAL TRANSDUCTION, AND BRAIN FUNCTION

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Our research is focused on stem cells, on their contribution to tissue maintenance and regeneration, and on signals that control their division and fate. We generate animal models to study stem cells and their environment and to investigate the mechanisms that determine the division and differentiation choices of these cells. Our main interest is in stem cells of the adult brain. Continuous production of new neurons in neurogenic regions of the adult brain is important for learning and memory, neural tissue repair, and response to therapies. We work to understand how different factors, ranging from drugs and surgery to aging and disease, affect adult neural stem cells and their progeny. We also investigate interactions between different types of stem cells that help coordinate tissue growth and repair. Furthermore, we study the multifunctional signaling molecule nitric oxide (NO) for which we have demonstrated an important role in organism development and tissue differentiation. Finally, we work to translate the results we obtain with animals to human physiology and therapy.

Neural Stem Cells and Brain Disorders

Continuous production of new neurons is supported throughout life in several areas of the brain, among them the hippocampus, a brain region that is crucial for cognitive function. New neurons are produced from neural stem cells and their birth is the result of a complex cascade of events that include symmetric and asymmetric divisions of stem and progenitor cells, exit from the cell cycle, programmed elimination of a vast number of newborn cells, and continuous changes of their morphology; this cascade culminates with the young neuron establishing connections with other cells and becoming integrated into the preexisting neuronal circuitry. Production of new neurons from stem cells can be modified by a wide range of intrinsic and extrinsic factors, acting to increase (e.g., antidepressants and physical exercise) or decrease (e.g., stress, aging, and disease) hippocampal neurogenesis. These factors may affect any step of the differentiation

cascade or may converge on a few defined targets. We are working to unravel the basic rules and mechanisms controlling the quiescence, division, self-renewal, and differentiation of neural stem cells and their progeny.

To define the steps of the division/differentiation cascade and to determine the targets of important pro- or anti-neurogenic stimuli, we use transgenic reporter animal lines, often in combination with other genetically modified lines. We use the resulting compound lines to dissect the neurogenesis cascade into distinct stages and then to identify the steps that are specifically affected by a particular stimulus; this analysis has been complemented by computational modeling of the stem and progenitor cell dynamics, performed in continuous collaboration with Dr. Alex Koulakov at CSHL. This has allowed us to produce a comprehensive scheme of the neurogenic and astrogenic arms of the cascade. This detailed description of the hippocampal stem cell life cycle provides a conceptual framework with which to map the targets of various neurogenic stimuli and compare different strategies by which stem cells are harnessed for tissue regeneration.

We have already used this scheme to identify the targets of various antidepressant drugs and treatments, exercise, radiation, chemotherapeutic agents, trauma, ablation of dopaminergic neurons, and aging. In particular, we found that such diverse antidepressant therapies as fluoxetine, running, and deep brain stimulation of distant brain regions converge on the same step of the cascade and increase the number of divisions of the transit-amplifying progenitor population. Importantly, these treatments do not affect the pool of stem cells; in contrast, we found that electroconvulsive shock, a highly efficient antidepressant therapy, activates division of stem cells in the adult hippocampus.

We have also found that trauma and disease can activate stem cells of the hippocampus, even when distant regions of the brain are damaged. In particular, we found that moderate trauma of the cortex induces division of stem cells in the adult hippocampus. Furthermore, we found that in the mouse model of Parkinson's

disease, ablation of dopaminergic neurons of the substantia nigra can transiently activate division of stem cells in the hippocampus (which receives dopaminergic inputs from the substantia nigra). We also found that L-DOPA, used in the clinical treatment of Parkinson's disease, does not alter the effect of neuronal ablation on cell division in the hippocampus. Together, our results suggest that even distant events which do not directly impact the hippocampal area, such as cortical trauma or ablation of midbrain neurons, can activate neural stem cells of the hippocampus. Such activation may reflect the induction of innate repair and plasticity mechanisms by the injured brain; however, it may also potentially lead to a premature exhaustion of the neural stem cell pool, particularly in the aging organism.

Our results also shed light on the age-related decrease in the production of new neurons. This age-related decline in the number of new neurons is profound and has been observed across mammalian species. Given the significance of new neurons for cognitive function, it is conceivable that reduced neurogenesis contributes to age-related cognitive impairment. We found that age-related decrease in hippocampal neurogenesis under normal conditions is driven by the disappearance of neural stem cells via their conversion into mature hippocampal astrocytes. Importantly, this astrocytic differentiation is coupled to a rapid succession of asymmetric divisions of the activated stem cells. Thus, in contrast to the conventional model of multiple cycles of activation and quiescence of stem cells, hippocampal neural stem cells, once activated, leave the stem cell pool. We described the life cycle of an adult neural stem cell and proposed a "disposable stem cell" model which posits that an adult hippocampal stem cell is quiescent throughout the entire postnatal life, but, when activated, it undergoes several rapid asymmetric divisions (eventually producing a neuron) and then exits the pool of stem cells by converting into an astrocyte. This model reconciles observations on the age-related decrease in production of new neurons, the age-related increase in astrocyte number, the disappearance of hippocampal neural stem cells, and the remodeling of the neurogenic niche. Together, these continuous changes underlie age-dependent decrease in production of new neurons and may contribute to age-related cognitive impairment.

With the increasing evidence that adult neurogenesis is important for cognitive function and mood regulation, the ability to detect variations in the level of neurogenesis in the live brain becomes important for diagnostic purposes. In a broad collaboration with Dr. Helene Benveniste (Stony Brook University and Brookhaven National Laboratory), we are working to characterize changes in magnetic resonance spectra in the live human and animal brain that would be indicative of the changes in neurogenesis. By modifying and adjusting widely used methods of analyzing in vivo spectra, we are able to reliably detect indicative signals in the live animal brain under normal conditions and after electroconvulsive shock. Moreover, this approach is now being investigated in preclinical and clinical trials and may become useful for following neurological and psychiatric disorders and cancer.

Stem Cells in Nonneural Tissues

Adult tissues undergo continuous cell turnover in response to stress, damage, or physiological demand. New differentiated cells are generated from dedicated or facultative stem cells or from self-renewing differentiated cells. Adult stem cells are located in specialized niches that restrict their division and support their undifferentiated status. Some time ago, we found that the same reporter lines that we developed and used to identify stem cells of the brain can also help to highlight stem and progenitor cells in a range of other tissues. This list is not limited to cells of neural origin; it now includes stem cells of such dissimilar structures as brain and spinal cord, hair follicles, liver, pancreas, skeletal muscle, testis, ciliary margin of the eye, anterior pituitary, skin, and bone marrow. This observation provides a means of isolating adult stem and progenitor cells, tracing their lineage, and studying mechanisms controlling their quiescence, division, self-renewal, and differentiation.

In an important application of this reporter-based approach, in collaboration with Dr. Paul Frenette, we identified mesenchymal stem cells in bone marrow and demonstrated that they form a niche for hematopoietic stem cells. These two types of stem cells are found in close association; furthermore, mesenchymal stem cells express high levels of genes that are important for hematopoietic stem cell maintenance and, when depleted, result in a reduced ability of hematopoietic stem cells to home in the brain marrow. This is the first example of a partnership between two distinct somatic stem cell types, suggesting that such heterotypic stem cell pairs may exist in other tissues.

NO, Development, and Differentiation

Much of our interest is related to a signaling molecule, nitric oxide (NO). NO has been extensively character-

ized as an indispensable regulator of vasodilation, neurotransmission, immune response, and cell death. Throughout the last two decades, we have demonstrated that in several developmental and differentiation contexts, ranging from differentiating neurons to developing fruit flies and frogs, NO helps to control the balance between proliferation and differentiation by suppressing cell division. For instance, it acts as a negative regulator of cell division in the developing and adult nervous system, such that by manipulating NO levels, we can change the number of neural stem and progenitor cells. Furthermore, we found that the neuronal NO synthase isoform (nNOS), usually associated with brain function, regulates hematopoiesis in vitro and in vivo: nNOS is expressed in stromal cells and produces NO which acts as a paracrine regulator of hematopoietic stem cells. Thus, NO acts as a regulator of stem and progenitor cell activity and cell differentiation in a range of tissues.

Continuing to explore the diversity of NO's biological functions, we found that NO also acts as a regulator of morphogenesis during early development by coordinating two major morphogenetic processes, cell division and cell movement, through two overlapping but separate signaling pathways. Inhibition of NO synthase during early development of *Xenopus* increases proliferation in the neuroectoderm and inhibits convergent extension in the axial mesoderm and neuroectoderm, indicating that during development, NO suppresses division and facilitates movement of cells. Such concurrent control by NO helps ensure that the crucial processes of cell proliferation and morphogenetic movements are coordinated during early development.

We have now discovered yet another role of NO and nNOS during early development—its function as a regulator of planar polarity of cilia growth and function in the mucosecretory epithelium, as exemplified by the skin in *Xenopus* larvae and the airway epithelium in mammals. In these tissues, the spatial organization of cilia is important for the polarized beating of cilia and the directional distribution of mucus. We demonstrated planar polarity of the distribution of cilia in the *Xenopus* larvae and found that NO and XNOS1 (an ortholog of nNOS) are important mediators of this polarity. We also found a new mode of establishing planar polarity by function-dependent stabilization of cilia growth and determined the signaling pathway that links the XNOS1 activity to ciliogenesis and cilia polarity. Moreover, we have now extended these observations to the tracheal and bronchial epithelium of mammals; these results indicate a conserved evolutionary role for NO in the regulation of cilia structure and function and highlight the role of NO in human health and disease.

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MOLECULAR ANALYSIS OF NEURONAL RECEPTORS AND ION CHANNELS

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                   P. Singh

The research in my group is aimed at understanding the molecular basis for the function of cell surface proteins that trigger cellular signal transductions involved in neurotransmission in the mammalian brain. It is hoped that this work will help in the design of compounds with therapeutic potentials in neurological diseases and disorders including schizophrenia, depression, stroke, and Alzheimer's disease. Toward this end, we are conducting structural and functional studies on neurotransmitter receptor ion channels and intramembrane enzymes, which facilitate and regulate the strength of neurotransmission. To achieve our goals, we use X-ray crystallography to determine three-dimensional structures of target proteins and test structure-based mechanistic hypotheses by site-directed mutagenesis in combination with biochemical and biophysical techniques including electrophysiology. Our group is currently focused on understanding the structure and function of three classes of cell surface proteins, *N*-methyl-D-aspartate receptor (NMDAR) and γ-secretase, dysfunctions of which are highly implicated in the pathogenesis of neurodegenerative diseases including Alzheimer's disease.

Structure and Function of NMDARs

NMDARs belong to the family of iGluRs, which mediate the majority of excitatory synaptic transmission in the mammalian brain. Dysfunctional NMDARs are implicated in neurological disorders and diseases including seizure, stroke, schizophrenia, Parkinson's disease, and Alzheimer's disease. NMDARs are multimeric ligand-gated ion channels composed of the GluN1 and GluN2 subunits, which bind glycine and L-glutamate, respectively. Unlike non-NMDARs that can form functional ion channels as homotetramers, the NMDARs can only function as heteromultimers composed of GluN1 and GluN2, and opening of the ion channels requires concurrent binding of both

glycine and glutamate to GluN1 and GluN2, respectively. The four distinct GluN2 subunits define pharmacological subtypes of NMDARs that differ in ion channel properties, ligand specificity, and spatial and temporal expression patterns. Subtype-specific regulations of NMDARs have shown some neuroprotective effects and therefore are considered an effective strategy to treat neurological disorders and diseases. There has been an enormous enthusiasm to understand the molecular basis for subtype specificity in NMDARs and to create subtype-specific compounds that can target specific neuronal circuits.

The NMDAR subunits are modular proteins composed of the amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TM), and carboxy-terminal domain (CTD) (Fig. 1). The focus of our study in 2010 was LBD of the GluN2D subunit, which binds to agonists including L-glutamate and NMDA. GluN2D is a predominant subtype of NMDARs that are expressed during the early developmental stage of the brain. The GluN2D NMDARs are also distinct from other subtypes of NMDARs in that they elicit significantly slow deactivation and have low open channel probability. To understand the molecular basis for the pharmacological properties unique to the GluN2D receptors, we have solved the crystal structures of GluN2D LBD in complex with L- and D-glutamate, L-aspartate, and NMDA and finished refinement of the structural models for all of the forms (Fig. 2). The GluN2D LBD has an overall clamshell-like architecture composed of domain 1 (D1) and domain 2 (D2), similar to the GluN2A LBD previously solved.

All of the agonists bind to the interdomain cleft between the D1 and D2 domains and appear to stabilize closed clamshell conformation. Importantly, this is the first study that successfully visualizes the mode of binding of an NMDA molecule; therefore, we can assess the mechanism of NMDA subfamily specificity. Binding of NMDA involves displacement of a water molecule (W
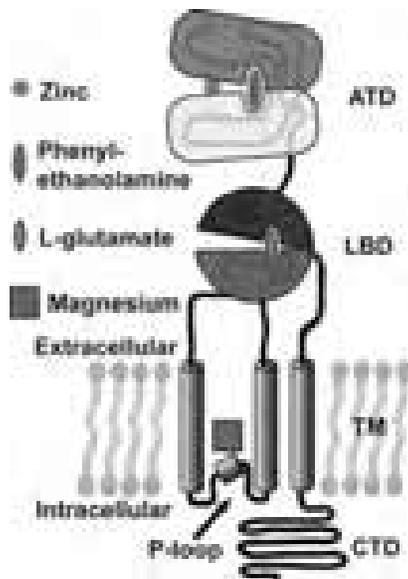
Figure 1. Domain organization of NMDAR subunit. The amino-terminal domain (ATD) at the extracellular region is followed by S1, transmembrane region (TM) 1 and 2, P-loop, the S2 segment, TM3, and carboxy-terminal domain (CTD). The S1 and S2 peptides form a ligand-binding domain (LBD) that binds agonists and antagonists. ATD in NMDARs bind allosteric modulators (zinc and phenylethanol-amines). TM forms the ion channel in the context of the heteromultimeric assembly of GluN1 and GluN2 subunits, which is blocked by magnesium at resting membrane potential. CTD interacts with intracellular proteins such as PSD95 and CAMKII to mediate cellular signal transduction important for synaptic plasticity.

One intriguing aspect of the GluN2D LBD structures was that a region of the structure, which we call the hinge loop, located at the back side of the ligand-binding site, is different between the L-glutamate-bound form and all the other forms. That led us to two hypotheses: (1) L-glutamate uniquely activates and de-activates the GluN1/GluN2D NMDARs, and (2) structural variability around the hinge loop may be responsible for regulating speed of deactivation. We have tested these hypotheses by using an electrophysiological approach in collaboration with Stephen Traynelis at Emory University. We have made a series of chimera receptors between GluN2A and GluN2D within the LBD and measured deactivation speeds by the patch-clamp method coupled to fast perfusion. GluN2A was used as a chimera partner because it mediates fast de-activation (~50 msec) compared to GluN2D (~3000 msec). The chimeric receptors tested, so far, are as follows: (1) GluN2A (GluN2D LBD), (2) GluN2A (GluN2D D1), and (3) GluN2A (GluN2D D2). As shown in Figure 4, all of the chimeras have substantially slow deactivation compared to the wild-type GluN2A. This tentatively indicates that both D1 and D2 are important in regulating deactivation speeds and that the GluN2D LBD domains facilitate slower deactivation speed compared to GluN2A LBD.

in Fig. 3A) by the *N*-methyl group whose placement is favored by the surrounding hydrophobic residues, Tyr-755 and Val-759. The equivalent residues for Tyr-755 in the AMPAR (α-amino-3-hydroxy-5-methyl-4-isox-azole proprionic acid receptors) and kainate receptor are leucine and methionine, respectively, pointing away from the binding pocket (Fig. 3C). The corresponding residues for Val-759 are methionine in AMPARs and threonine in kainate receptors, which are more hydrophilic (Fig. 3C). Furthermore, Asp-756 "folds" away from the binding pocket so that its β-carboxyl group does not interfere with the placement of the *N*-methyl group of NMDA. The aspartate residue at the 756 position is conserved among the subunits of NMDARs. In non-NMDARs, the equivalent residue of Asp-756 is glutamate, whose longer side chain would collide with the *N*-methyl group and disallow the placement of an NMDA molecule in the binding pocket.

Figure 2. Refined crystal structure of GluN2D LBD. The structure is clamshell-like composed of domain 1 (D1) and domain 2 (D2). Agonists including NMDA (stick) bind at the interdomain cleft (between D1 and D2). Glycine-threonine linker used to tether S1 and S2 peptides is shown as gray sphere.
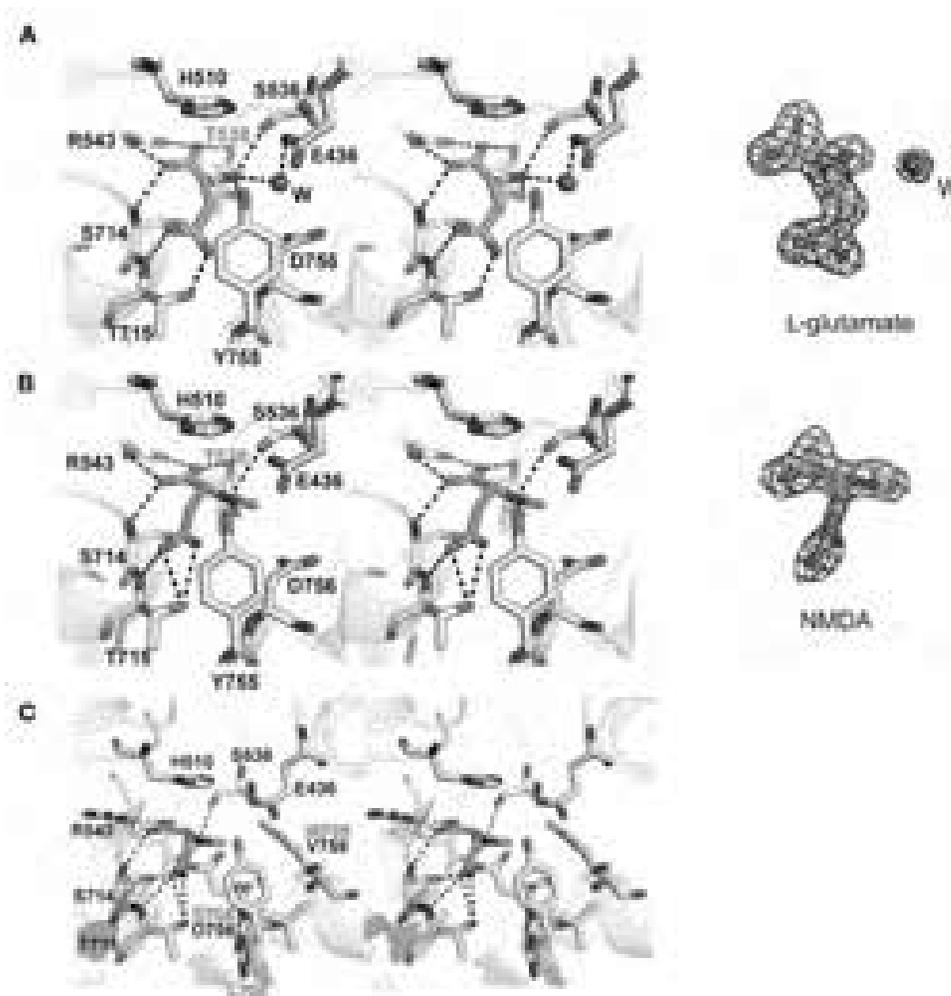
Figure 3. Ligand-binding site of GluN2D LBD in stereoview. (*A,B*) The GluN2D LBD structures in complex with L-glutamate and NMDA. Shown on the right-hand side in mesh are electron density maps contoured at 4σ. (*C*) Orientation of nonconserved residues in GluA2 LBD.

Structure and Function of γ-Secretase

γ-Secretase is a multimeric protein complex of four transmembrane proteins including presenilin, nicastrin, APH-1, and PEN-2. Together, the complex mediates a molecular event called regulated intramembrane proteolysis, which is a type of proteolysis that occurs within the membrane. Intramembrane proteases are involved in numerous cellular processes including cholesterol homeostasis, development, immunosurveillance, and Alzheimer's disease.

γ-Secretase is an aspartyl-protease that cleaves various single-transmembrane substrates. One of the most well-studied substrates is amyloid protein whose cleaved fragment diffuses out of the membrane, forms β-amyloid

oligomers and fibril, and initiates signaling cascades resulting in neuronal cell death. Deposition of β-amyloid fibril is one of the hallmarks of the brain affected by Alzheimer's disease. However, mechanistic understanding of activities in γ-secretase and intramembrane proteases in general is restricted due to limited numbers of available molecular structures. The structural information of γ-secretase is currently limited to low-resolution images obtained from a single-particle analysis using cryoelectron microscopy. Our group is interested in revealing atomic structures of γ-secretase to gain insights into intramembrane proteolysis, substrate specificity, inhibition, and functional modulation at the molecular level. Currently, we are working toward understanding the mechanism of substrate recognition mediated by one of the γ-secretase components, nicastrin.
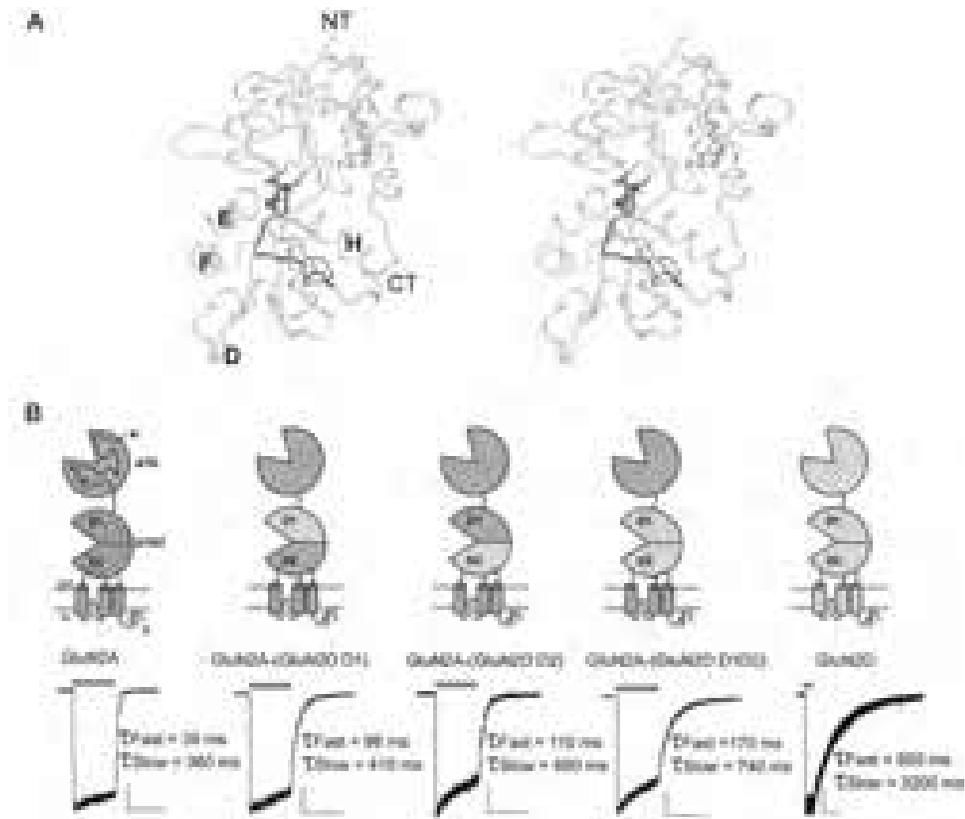
Figure 4. Roles of hinge loop. (*A*) Structures of GluN2D LBD in complex with various ligands. Note that the conformation of the L-glutamate-bound structure (cyan) is different from all the others. (*B*) Design of chimeric receptors between GluN2A and GluN2D and deactivation speeds measured by whole-cell patch clamp. The horizontal scale bar represents 2 sec for all traces, and the vertical scale bar represents ~200 pA for all traces.

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CONSTRUCTION AND FUNCTION OF GABA INHIBITORY CIRCUITS IN NEOCORTEX

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

Genetic Dissection of the GABAergic System in the Neocortex

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

Genetic Analysis of Chandelier Cells during Cortical Circuit Assembly

We have genetically captured chandelier cells (CHCs), the most distinctive class of cortical interneurons. CHCs exclusively innervate pyramidal cells at axon initial segments, the site of action potential generation. CHCs may thus exert decisive control over pyramidal cell firing and thereby dynamically configure neural ensembles. Because of their exceptional stereotypy and specificity, genetic capture of CHCs establishes a powerful experimental paradigm for studying their entire developmental history, from origin to function in cortical circuits. We have found that CHCs undergo massive pruning in both cell numbers and axons before the emergence of characteristic chandelier arbors in the third postnatal week. Conserved from mouse to humans, CHCs have been implicated in several brain disorders including schizophrenia and epilepsy. Alteration of CHCs, either as a direct consequence of mutations or indirectly through developmental compensation and maladaptation, is likely a hallmark and a sensitive probe for detecting aberrant cortical circuits in mouse models. Genetic analysis of CHCs not only will provide a key entry point to understanding the assembly and function of cortical circuitry but will also shed light on the pathogenic mechanisms of neuropsychiatric disorders.

Role of GABA Transmission Activity-Dependent Development of Inhibitory Synapses

The synaptic connectivity of GABAergic interneurons in the neocortex is often characterized by both specificity and robustness. For example, basket interneurons selectively target the soma and proximal dendrite (i.e., perisomatic region) of pyramidal neurons, and a single basket cell innervates hundreds of pyramidal neurons with tens of synapses on each target cell. Such specific and exuberant patterns of synaptic innervation likely contribute to the effective control of the output and synchrony of pyramidal neurons, but the underlying developmental mechanisms are largely unknown. In the past year, we have made significant progress in understanding how GABA signaling, presynaptic $GABA_B$ receptors, and neurexin (NRX)–neuroligin synaptic adhesion molecules contribute to GABA-mediated regulation of inhibitory

synapse development. We found that developing basket cell axons and presynaptic boutons are highly dynamic. Interfering with GABA$_B$ receptor function by either antagonists or single-cell knockout destabilizes nascent GABAergic terminals. These results suggest that local activation of presynaptic GABA$_B$ receptors stabilizes nascent synaptic contacts and contributes to activity-dependent regulation of inhibitory synapse development. NRXs and neuroligins are key synaptic adhesion molecules that also recruit synaptic signaling machineries. NRXs consist of α and β isoforms, but how they couple synaptic transmission and adhesion to regulate activity-dependent synapse development remains unclear, in part due to poor understanding of their cell biology and regulation in the relevant neurons. Here, we examined the subaxonal localization, dynamics, and regulation of NRX1α and NRX1β in cortical perisomatic inhibitory synapses. Both isoforms are delivered to presynaptic terminals but show a significant and different turnover rate at the membrane. Although NRX1α is highly diffuse along developing axons and filopodia, NRX1β is strictly anchored at terminals through binding to postsynaptic ligands. The turnover rate of NRX1β is attenuated by neural activity and presynaptic GABA$_B$ receptors. NRXs are thus intrinsically dynamic, but they are stabilized by local transmitter release. Such an activity-adjusted adhesion system seems to be ideally suited to rapidly explore and validate synaptic partners guided by synaptic transmission.

Impact of GABAergic Deficiency in Synaptic Transmission and Behavior

A highly reproducible observation in schizophrenia (SZ) is the reduction of mRNA for GAD67—the principle enzyme for GABA synthesis in cortex—in certain types of cortical interneurons, particularly those expressing parvalbumin (PV). GAD67 expression is regulated by cellular activity, and its reduction in schizophrenia is most likely a downstream impact of reduced engagement of the PV cell network. Because PV cells have an important role in the generation and maintenance of γ wave activity, dysfunction in these cells is thought to contribute to cognitive deficits in SZ, such as working memory impairment. However, there are no effective treatments to target GABA-system dysfunction in schizophrenia or to limit cognitive deficits in the disease. To model the cell-type-specific changes observed in SZ, we used a cre/lox system in mice to conditionally knock down *GAD67* in PV cells. Virus-vector-mediated green

fluorescent protein (GFP) expression was used for paired recordings from PV to pyramidal cells and for behavioral tests, including Y-maze spontaneous alternation to assess working memory function.

In young mice, reduction of GAD67 in PV cells led to weakened synaptic inhibition. Removal of one allele of *Gad1* (heterozygote) led to a 33% reduction of GAD67 in PV cells and further caused a supralinear deficit in synaptic transmission. Young mutant mice also demonstrated a behavioral phenotype, with reduced spontaneous alternation in the Y-maze. Additionally, pyramidal cells demonstrated hyperactive spiking properties in response to current injection. In adult mice, however, both the synaptic and behavioral deficits had resolved, leading the mutant mice to be indistinguishable from controls in all measured parameters. We therefore have demonstrated that GABA deficiency does in fact lead to weakened synaptic transmission, with a concurrent behavioral phenotype. However, weakened inhibition in juvenile mice appears to not produce any irreversible changes, as homeostatic mechanisms were able to fully eliminate the impact of this artificially induced GAD67 deficit. Our results from this rodent study support that PV cell function, or possibly GAD67 expression level itself, presents a possible treatment target for cognitive dysfunction.

Cell-Type-Based Epigenomic Analysis of GABAergic Neurons

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

microRNAs (miRNAs) regulate gene expression in a sequence-specific manner and are implicated in development and function of mammalian brain, but the cellular heterogeneity impedes in-depth studies of miRNA. We have established a cell-type-based "miRNA-tagging" affinity purification method using a Cre-loxP binary system in genetically engineered mice. We have demonstrated the success of this method by profiling miRNAs in major types of neurons in the mouse neocortex and cerebellum using deep sequencing. We have discovered highly distinct profiles of miRNA expression in different cell types, novel miRNAs, and novel features of miRNAs biogenesis. These tools will facilitate the functional studies of miRNAs in the brain.

A Novel Function of MeCP2 in mRNA Translation

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

GABAergic Interneurons and Neurodevelopmental Disorders

We are studying alterations of GABAergic inhibitory circuits in mouse models of RTT, one of the autism spectrum disorders, caused by mutations in the X-linked gene *MeCP2*. Our general hypothesis is that *MeCP2* mutations perturb the postnatal maturation of the connectivity, function, and plasticity in subsets of inhibitory interneurons in distributed brain areas, leading to altered development and maladaptive plasticity

of neural circuits, and characteristic behavioral deficits.

Neuronal circuits in the brain are shaped by experience during "critical periods" in early postnatal life. In the cortex, this activity-dependent development is coincident with the functional maturation of parvalbumin (PV[+]) GABAergic interneurons, although the underlying mechanisms are unclear. We found that loss of methyl-CpG-binding protein (MECP2) results in accelerated maturation of PV[+] fast-spiking cortical interneurons, likely resulting in a shift in critical period onset using molecular markers and physiological parameters. Furthermore, loss of *MeCP2* in PV[+] interneurons resulted in increased expression of the rate-limiting enzyme GAD67, which synthesizes GABA, the major inhibitory neurotransmitter in the brain. Previous studies from our lab have provided evidence for the role of GABA signaling not only in mediating inhibitory transmission, but also in regulating activity-dependent maturation of inhibitory synapses and innervation patterns. Our current results indicate a requirement for *MeCP2* in regulating the development of activity-dependent maturation of PV[+] GABAergic interneurons via GAD67, which in turn innervate the perisomatic region of pyramidal neurons and control their output. As *MeCP2*-null mice serve as a model for RTT, a devastating neurodevelopmental disorder, our findings provide insight into the inappropriate synaptic plasticity underlying the pathogenesis of this disease.

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NEUROBIOLOGY OF COGNITION AND DECISION MAKING

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                 B. Hangya   H.J. Pi   J. Sanders
                 J. Hirokawa S. Ranade

We are interested in identifying the neurobiological principles underlying cognition and decision making. We use a reductionist approach, distilling behavioral questions to quantitative behavioral tasks for rats and mice that enable us to monitor and manipulate the neural circuits supporting behavior. Using state-of-the-art electrophysiological techniques, we first seek to establish the neural correlates of behavior and then use molecular and optogenetic manipulations to systematically dissect their underlying neural circuits.

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

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

Role of Orbitofrontal Cortex in Confidence Judgments

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

If you are asked to evaluate your confidence in your decision—how sure are you that you made the correct choice—you can readily answer. What is the neural basis for such judgments? Is knowledge about beliefs an example of the human brain's capacity for self-awareness? Or is there a simpler explanation that might suggest a more basic yet fundamental role for uncertainty in neural computation? Previously, we discovered neurons in orbitofrontal cortex (OFC) that signal decision confidence. We are pursuing these initial observations by trying to establish that confidence-related neural activity in the OFC is *causally required for* confidence judgments.

First, we established a new olfactory decision task for rats, in which an estimate of decision uncertainty or confidence about the original decision is turned into a behavioral action. We designed a task in which we could measure confidence behaviorally on a trial-by-trial basis in order to examine the neural mechanisms of confidence judgments. To manipulate confidence, we used an olfactory mixture categorization task in which we can systematically vary decision uncertainty by changing the ratio of the two components in the odor mixture. To measure confidence, we delayed reward delivery after correct choices and measured the time an animal was willing to wait at the reward ports. This task allows us to examine how the timing of the decision to leave the reward port ("abort decision") depends on the uncertainty about the original decision.

Although we observed neural correlates of confidence in OFC, it may be one of several nodes in a network subserving confidence-guided decisions. Interestingly, however, several studies have shown that humans with OFC lesions are insensitive to the degree of uncertainty, indicating that the OFC may be causally involved in some uncertainty-guided behaviors. Therefore, we hypothesized that inactivating OFC leaves the odor-guided decision intact while impairing the use of confidence-guided abort decisions. To test this, we bilaterally implanted double cannulae to temporary blockade neural activity in OFC. We found that inactivation of OFC disrupted the dependence of waiting time on decision confidence without changing the accuracy of the sensory decision. These results establish, for the first time, that there is an anatomical locus for confidence and that confidence reports and the computation of decisions are distinct processes localized to different brain regions.

If OFC activity directly controls abort decisions, then activation of the relevant neurons should trigger aborting in rats. How might OFC activity directly influence behavior? We are currently testing the hypothesis that a distinct output pathway from OFC is responsible for initiating abort decisions. We use a viral strategy to retrogradely deliver ChR2 to OFC neurons from several known target areas with motor functions, such as the striatum and the periaqueductal gray. Our ultimate aim is to define a precise neural circuit for computing and using decision confidence.

Confidence Judgments in Humans

J. Sanders

Methods for measuring decision confidence in humans have traditionally been limited to various forms of verbal reports and postdecision wagers. Our goal was to develop a new confidence-reporting task that provides both implicit and explicit measures of decision confidence and is suitable for both rodents and humans in order to make direct comparisons.

Toward this goal, we developed an auditory stereo click train discrimination task in which subjects are asked to report the side with the higher click rate. Our implicit measure of confidence is based on the notion that when confident, it makes sense to wait longer for an uncertain reward. Preliminary results demonstrate that both humans and rats optimize their decision-making strategies in this task by adjusting this waiting time measure to match the quality of sensory evidence. In humans, we can also prompt subjects for explicit self-reports of confidence (1–5 scale), and these correlate with the implicit waiting time measure.

On the basis of these results, we are now in a position to use these quantitative measures of decision confidence for human studies, through collaborations, using neuroimaging and genetic analysis. Aside from its usefulness in human studies, 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.

Auditory Sequences and Novelty Processing in Hippocampus

J. Sanders

The hippocampus is thought to have a key role in episodic memories, those linked to a specific time and place, as well as in encoding the temporal order and familiarity of events. Electrophysiological studies in the rodent hippocampus have traditionally focused on the spatial correlates of neurons, characterizing them as "place cells," which represent unique spatial locations in space. Although these neurons are known to exhibit a number of nonspatial correlates as well, their responses to abstract nonspatial attributes remain poorly understood.

We are studying the nonspatial correlates of hippocampal neurons in head-fixed mice trained to listen for a target sound following a sequence of eight unique, natural sounds. We record single units from the CA1 subfield of hippocampus while head-fixed mice listen to the familiar sequence, novel sequences, and a hybrid sequence (familiar order, but interrupted by novel elements). We noted that many CA1 cells have short-latency responses to sound onsets and signal abstract attributes not explicitly present in the sounds themselves: temporal context, reward cues, and reward outcome.

These results show that in a head-fixed setting, the awake rodent hippocampus rapidly encodes abstract properties of auditory events. Because most recordings from rodent hippocampus describe neurons as "place cells," we are currently comparing the response of sound-selective neurons to spatial cues. We expect that these results will open a new avenue for exploring nonspatial computations in the hippocampus.

Neural Circuit Dynamics of Genetically Identified Interneuron Types in Behaving Mice

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

The medial prefrontal cortex (mPFC) in humans and rodents has been implicated in a variety of goal-directed behaviors, including working memory, inhibitory response control, and attentional set switching. Electrophysiological recordings from the mPFC show great diversity of neuronal responses to specific behavioral variables. On the other hand, we know that the mammalian cortex is composed of variety of cell types among which GABAergic interneurons exhibit the largest diversity in connectivity, morphology, and intrinsic physiology. Does the anatomical and molecular variety of interneuronal types map onto the diversity of neuronal responses in behaving animals? To answer this question, we developed a method to electrophysiologically record freely behaving animals from a genetically

defined neural population. Our method relies on combining optogenetic tools with behavioral electrophysiology. We focused on somatostatin (SOM)- and parvalbumin (PV)-expressing interneurons that target, respectively, the distal dendritic and perisomatic regions of pyramidal cells and are thought to have complementary functions in regulating principal cell firing. We used a simple auditory-cued reward anticipation task where mice are trained to run back and forth on a linear track and collect water rewards at the end of the track by nose poking in the water port.

We found that the majority of PV and SOM neurons showed distinct and homogeneous response profiles to behavioral periods when animals were initiating the runs on a linear track shortly after reward consumption. PV neurons abruptly ramped up their activity while SOM neurons slowly decreased firing. We also observed that artificially activating groups of PV and SOM neurons provide respectively short and long inhibition to putative pyramidal cells, suggesting that these two groups of interneurons are temporal specialists for inhibition.

The homogeneity of responses within PV and SOM interneurons and their "inhibitory footprint" has strong implications for mPFC circuit dynamics. We hypothesize that SOM neurons are in a position to gate intracortical inputs to layer-5 pyramidal cells. On the other hand, fast and homogeneous modulation of PV interneuron firing might provide a "reset signal" for ensembles of pyramidal cells by synchronizing their activity.

Function of VIP Interneurons in Cortical Microcircuits

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

We are interested in understanding how the diversity of cortical inhibitory interneurons underlies distinct neural circuit dynamics that direct perception and behavior. As part of this project, we have begun to study vasoactive intestinal peptide-expressing (VIP) interneurons. Although VIP neurons constitute less than 2% of the total cortical neuronal population, previous studies indicate that VIPs might have a distinct function in the local cortical column. VIP neurons are mostly bipolar, and their processes are vertically oriented. Interestingly, these neurons specifically target other inhibitory neurons and avoid pyramidal cells. On the basis of these ideas, we are currently testing the hypothesis that VIP inhibitory interneurons provide local excitation to a patch of cortex via disinhibition of other interneurons.

Neural Representation of Social Decisions and Rewards

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

Mice, like humans, are social animals. To interact, cooperate, and compete with others, mice have to collect information about each others' identity, fertility, and likely intent. We want 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. These circuits tend to be shallow, from sensory input to motor actions, and highly stereotyped, enabling the systematic dissection of this system.

We have begun our studies by recording from the medial amygdala, the first brain region that combines information from the two olfactory systems, the main olfactory system transducing volatile odors and the vomeronasal system detecting behaviorally significant chemical cues, such as pheromones. To deliver social stimuli and determine their "value" for a mouse, we developed a new behavioral setup—the "mouse carousel." This system will improve stimulus delivery precision and repertoire. In the future, we hope to find molecular markers that will enable us to identify and record from a specific population of neurons in the medial amygdala.

Addicted to Light: Optogenetic Control of Dopamine during Behavior

S. Rengarajan

Dopaminergic neurons in the ventral tegmental area (VTA) have been implicated in driving learning and reward-seeking behaviors. Electrophysiological recordings show that these neurons signal the discrepancy between reward prediction and outcome, the "prediction error signal," a theoretically important variable for learning. However, it is not known whether prediction error signals have a causal role in changing behavior.

To test this idea, we are using an optogenetic approach in behaving mice. We are using a two-choice discrimination paradigm to test whether prediction error signals influence updating in subsequent trials. To control VTA firing, we implant fiberoptic probes in TH-cre mice expressing either ChR2 or Arch to respectively activate and suppress dopamine neuron firing. Our hope is to artificially induce behavioral updating by optical activation and suppress normal updating by optical inhibition of dopaminergic neurons.

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

S. Ranade, H. Retallack, S. Rengarajan, J. Woldenberg, B. Hangya

The cholinergic basal forebrain (CBF) is a vitally important yet poorly understood neuromodulatory system that is thought to have significant roles in cognitive functions including attention. Projections of CBF innervate the entire cortical mantle and release acetylcholine, which enhances cortical processing. Although lesion studies and pharmacology have delineated a role for acetylcholine in attention, these techniques are lacking in spatial, temporal, and neurochemical specificities. The goal of this project is to understand the function of the CBF in attention, using a powerful combination of molecular genetic, electrophysiological, optogenetic, and psychophysical techniques.

We use a knockin mouse line (Chat-cre) to specifically target cholinergic neurons in the basal forebrain. We can express optical activators/inactivators delivered via viral vectors specifically in these neurons to manipulate their activity with high spatiotemporal precision. We have successfully developed visual and auditory versions of sustained attention tasks in mice. In this task, mice report the occurrence or nonoccurrence of a rare and unpredictable sound/light flash at two different locations to receive water. Performance of the task is an indicator of attention. We are currently testing the causal role of acetylcholine in this task by transiently inactivating cholinergic neurons with light during behavior. We also plan to record the activity of "optogenetically tagged" cholinergic neurons in mice engaged in this task to understand the response dynamics of these neurons. In general, these tools enable an unprecedented degree of mechanistic investigation of the behavioral functions of the CBF system and provide an entry point for future studies of cholinergic degenerative diseases, such as Alzheimer's disease.

Mechanisms of Cholinergic Modulation in the Auditory Cortex

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

Acetylcholine (Ach) has profound physiological effects on cortical neurons. For instance, electrical stimulation of the nucleus basalis leads to desynchronization of the cortical EEG. Cholinergic agonists increase firing rate, decrease firing correlations between neighboring neurons, and change the receptive field properties of cortical pyramidal neurons.

What is the physiological mechanism by which Ach modulates coritical neuronal firing? The goal of this project is to investigate the cellular and circuit mechanisms of cholinergic modulation of spontaneous and stimulus-evoked activity of cortical neurons in A1. We targeted the light activator, ChR2, to cholinergic neurons (see previous section) projecting to A1 to evoke light-triggered release of Ach. Using this technique, we observed the effects of Ach on auditory cortical responses. A high percentage of neurons increased their firing rate in response to light activation and some also showed changes in their auditory tuning curves. Using cell-attached recordings, we now plan to investigate the underlying cellular mechanism of cholinergic modulation. Furthermore, by recording from identified cholinergic neurons in awake mice, we would like to correlate their firing with network states of neurons.

Rapid Light-Induced Transcription in Mammalian Cells

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

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

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

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Duda Kvitsiani

THEORETICAL AND COMPUTATIONAL NEUROSCIENCE

A. Koulakov

Our laboratory develops mathematical models of neural processing. We work in parallel on three important topics. First, we are formulating a mathematical model for combining genetic information and experience (nature and nurture) during the development of neural connectivity. Our model describes how genes can help build neural networks and how neural activity adds a layer of plasticity to the network topology that reflects learning and experience. This model has been tested on simple circuits that are formed in the visual system and can be rewired using genetic, surgical, and pharmacological manipulations.

Second, we have been developing the neural network theory for olfactory processing. Our theory attempts to describe the olfactory space, which has been an elusive concept that excites the imagination of chemists, neuroscientists, and experimental psychologists alike. In our theory, we attempted to link several levels of olfactory perception: chemical, genetic, neural, and perceptual. Our main result so far has been the mapping between chemical and perceptual spaces that was established on the basis of a database of human olfactory responses. We have developed a robust description of the human perceptual olfactory space. This description is analogous to understanding the main directions in the human color space, i.e., red, green, and blue. These main perceptual dimensions can be understood in terms of properties of the underlying chemical compounds, at least, on the level of correlation. We also proposed a network theory for information processing in the olfactory bulb. We suggest that the granule cells, the inhibitory neurons of the olfactory bulb, form representations of smells using the network implementation of sparse overcomplete representations. Granule cells are remarkable because, unlike most of the neurons in the adult mammalian nervous system, they are continuously replaced by the new neurons produced from the neural stem cells.

Third, we have been working on the mathematical description of adult neural stem cell differentiation and proliferation in hippocampus.

These models can describe the evolution in time of various markers that experimental researchers use to study the division/differentiation cascade. By comparing the computational/theoretical models to experimental data, one can understand changes occurring in neurogenesis due to aging and antidepressant therapies.

Competition Is a Driving Force in Topographic Mapping

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

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

The Structure of Human Olfactory Space of Mixtures

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

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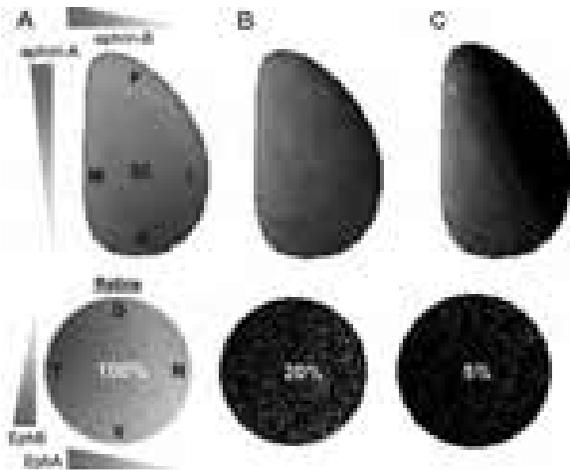
Figure 1. As the number of retinal cells is progressively decreased in the model, the projection from retina to the superior colliculus (SC) of the midbrain deforms until only a part of the target is filled with axons (*top right*). The case of ~5% of retinal cells present corresponds to the phenotype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

by Andrew Dravnieks. We have shown previously that 146D profiles of odorants that represent the responses of human observers to a variety of monomolecular odorants can be accurately described by a two-dimensional curved manifold. Here, we investigate the responses of human subjects to the set of 15 mixtures contained in the same database. The particular question that we address is whether the percepts of mixtures (the mixture space) reside close to the olfactory space determined from the monomolecular odorants. We show that indeed the mixture space can be predicted by the percepts of monomolecular odorants. The important difference, however, is that the mixture space contains one extra dimension compared to monomolecular odorants. Thus, approximating 146-dimensional profiles of monomolecular odorants with the two-dimensional curved manifold allows us to include 51% of variance contained in the data. In the case of mixtures, the same amount of variance can be explained by a three-dimensional curved space. One extra dimension, however, is predicted by the small residual fluctuations of monomolecular percepts around the two-dimensional curved monomolecular space. We conclude that the responses of human
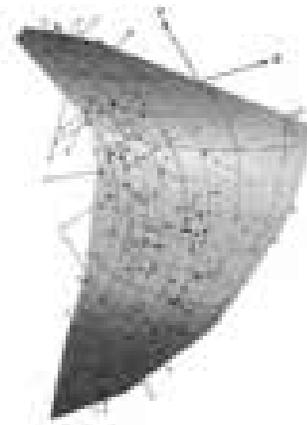
Figure 2. Structure of human olfactory space. Odors (crosses) cluster around a two-dimensional curved surface in the perceptual space. The two-dimensional surface exists in a 146-dimensional space of various perceptual descriptors. The parameters defining the positions on the surface (X and Y) are related to the molecule's chemical composition and hidrophobicity.

observers to mixtures can be found to be low dimensional and predictable from the percepts of monomolecular odorants.

Sparse Incomplete Representations: A Novel Role for Olfactory Granule Cells

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

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

tory code in the steady state and predicts that transient dynamics may be less sparse.

Disposable Tissue Hypothesis: A New Model for Hippocampal Neurogenesis

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

New neurons are continuously generated throughout the life of an animal in at least two areas of the mammalian brain: olfactory bulb and hippocampal dentate gyrus. Hippocampal neurogenesis dynamically responds to a multitude of extrinsic stimuli and may be important for behavior, pathophysiology, brain repair, and the response to drugs that modulate mood. New neurons are produced from a limited population of stem cells whose number declines with age. What factors determine the rate of decline in the hippocampal stem cell population? How can the rate of production of new neurons be varied? In this study, we have shown that soon after a stem cell is used for production of new neurons, it becomes an astrocyte, i.e., leaves the pool of neuroprogenitors. Therefore, one can think of the stem cells as disposable: Once they are used, they cannot be reused again. The decline in the number of stem cells therefore occurs in a use-dependent manner. Does

this mean that the more neurons produced, the faster the stem cells are lost? Not necessarily. The sequence of transformations that a cell must undergo before it becomes a new neuron is very complex. One of the steps involves an intermediate form of neuroprogenitors that are called amplifying cells. These cells divide rapidly, about once per day, to make more neurons. It turns out that antidepressant drugs, such as Prozac, affect the divisions of the intermediate amplifying cells without much impact on primary (disposable) stem cells. Together with experimental group of Grisha Enikolopov at CSHL, we have developed a computational model for the division/differentiation cascade that leads to the production of new neurons. Comparing the computational model to experimental results leads to detailed information about the changes occurring in the neurogenesis cascade due to antidepressant drugs, aging, and other therapies. Our approach will provide unique insights to the computational properties of the neural stem cells and their importance for mental health.

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

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

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

The Synaptic Circuitry of the Lateral Habenula and Behavioral Depression

The cellular basis of depressive disorders is poorly understood. Recent studies in monkeys indicate that neurons in the lateral habenula (LHb), a nucleus that mediates communication between forebrain and midbrain structures, can increase their activity when an animal fails to receive an expected positive reward or receives a stimulus that predicts aversive conditions (i.e., disappointment or anticipation of a negative outcome). LHb neurons project to and modulate dopamine-rich regions such as the ventral–tegmental area (VTA) that control reward-seeking behavior and participate in depressive disorders. Our study shows in two learned helplessness models of depression that excitatory synapses onto LHb neurons project-
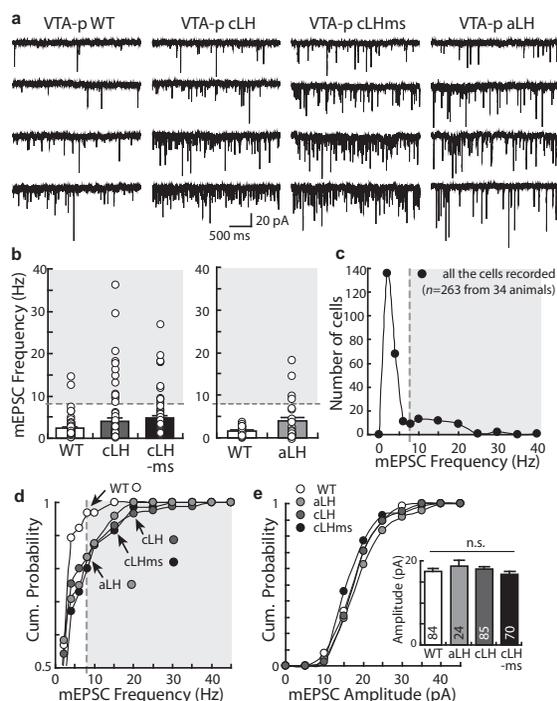
Figure 1. Increased excitatory synaptic transmission onto VTA-projecting LHb neurons in the learned helplessness models of depression. (*a*) Examples of mEPSCs recorded from VTA-projecting LHb neurons (VTA-p) from wild-type (WT), cLH, cLHms, and aLH animals. (*b*) Means (bars) and individual recordings (circles) of mEPSC frequency from VTA-projecting LHb neurons in different groups of animals. (*Left*) (WT) 2.4 ± 0.3, $n = 65$ (6 animals); (cLH) 4.0 ± 0.7, $n = 85$ (8 animals), $p < 0.05$, bootstrap; (cLHms) 4.7 ± 0.7, $n = 70$ (8 animals), $p < 0.001$, bootstrap. (*Right*) (WT) 1.8 ± 0.2, $n = 19$ (4 animals); (aLH) 3.9 ± 1, $n = 24$ (4 animals), $p < 0.05$, bootstrap. (*c*) Frequency distribution of mEPSC frequencies of all cells recorded exhibited bimodal distribution. (*d*) The cumulative probability of mEPSC frequency of VTA-projecting LHb neurons in different groups of animals ($p < 0.05$, K-S test comparing WT with any other groups). (*e*) The amplitude of mEPSC was not different among different animal groups ($p > 0.3$, bootstrap).
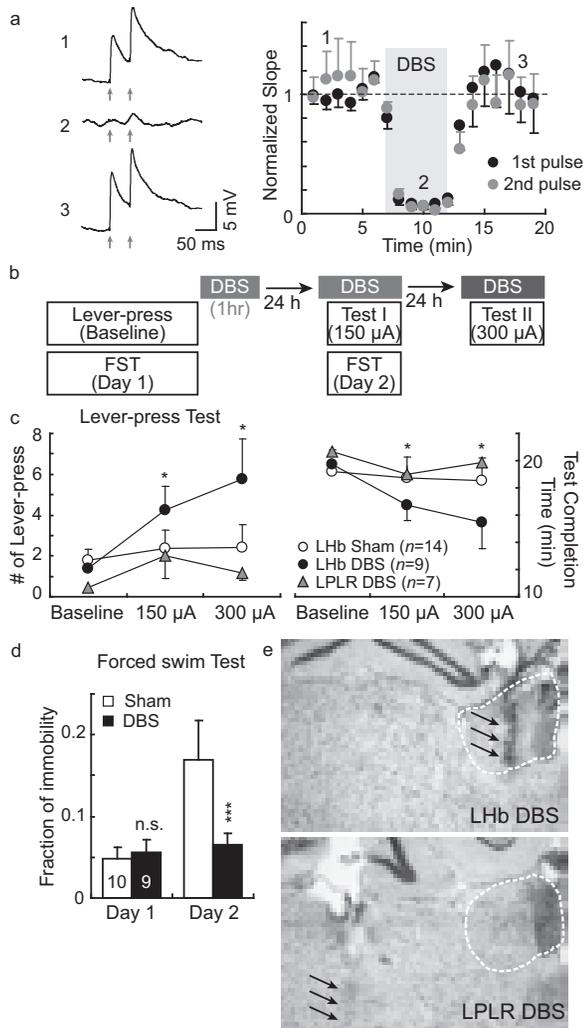
Figure 2. DBS in LHb suppresses excitatory synaptic transmission and reverses learned helplessness. (*a, Left*) Example EPSPs (paired-pulses) recorded from a VTA-projecting LHb neuron before (1), during (2), and after (3) stimulation mimicking DBS. Arrows indicate when paired-pulses were given. (*Right*) Average EPSP slope at indicated time points: (1) before (first pulse 1.1 ± 0.1; second pulse 0.9 ± 0.1; $n = 6$ [4 animals]); (2) during (first pulse 0.2 ± 0.07; second pulse 0.03 ± 0.03), $p < 0.001$ for both pulses compared with those in 1; (3) after (first pulse 1.2 ± 0.3; second pulse 0.9 ± 0.2) DBS. (*b*) A schematic diagram showing the experimental procedures. (*c*) Lever presses (*left*) and test completion time (*right*) for animals that received DBS or sham stimulation in the LHb, or DBS in the LPLR (lateral postthalamic nuclei, laterorostral), before (baseline), or after DBS of different intensities. DBS in LHb ($n = 9$): Lever press baseline 1.2 ± 0.4; 150 µA session 3.9 ± 1; 300 µA session ($n = 8$) 5.8 ± 2. Test completion time baseline 19.9 ± 0.4; 150 µA session 17.1 ± 1; 300 µA session ($n = 8$) 15.4 ± 2. Sham ($n = 14$): Lever press baseline 1.8 ± 0.5; 150 µA session 2.4 ± 0.9; 300 µA session 2.4 ± 1. Test completion time baseline 19.2 ± 0.5; 150 µA session 18.7 ± 0.9; 300 µA session 18.5 ± 1.1. DBS in LPLR ($n = 7$): Lever press baseline 0.4 ± 0.2; 150 µA session 2 ± 1; 300 µA session 1.1 ± 0.3. Test completion time baseline 20.6 ± 0.2; 150 µA session 19 ± 1.2; 300 µA session 19.9 ± 0.3. For DBS in LHb group, $*p < 0.05$ compared with baseline. Sham and DBS in LPLR group: $p > 0.05$ for both measurements at both sessions compared with baseline (bootstrap). (*d*) Immobility during FST. DBS Day 1: 0.06 ± 0.01, Day 2: 0.06 ± 0.01, $n = 9$; Sham Day 1: 0.05 ± 0.01, Day 2: 0.17 ± 0.05, $n = 10$; DBS vs. Sham in Day 2: $***p < 0.001$, bootstrap. (*e*) Representative cresyl violet staining of coronal brain sections; arrows indicate electrode track in the LHb (upper) or LPLR (lower). All error bars represent S.E.M.

ing to the VTA are potentiated (Fig. 1). Synaptic potentiation correlates with an animal's helplessness behavior and is due to an enhanced presynaptic release probability. Depleting transmitter release by repeated electrical stimulation of LHb afferents, using a protocol that can be effective in depressed patients, dramatically suppresses synaptic drive onto VTA-projecting LHb neurons in brain slices and can significantly reduce learned helplessness behavior in rats (Fig. 2). Our results indicate that increased presynaptic action onto LHb neurons contributes to the rodent learned helplessness model of depression.

Depression and Resilience: The Role of the Medial Prefrontal Cortex

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

To determine cellular activity in the mPFC in different groups of animals, we measured c-Fos expression in the infralimbic (IL) and prelimbic (PL) regions of the mPFC. We found that in the learned helplessness (LH) model of depression, neurons in the mPFC were activated. After the LH procedure, animal behavior diverged to become either depressed or resilient to depression. Interestingly, the resilient animals had significantly enhanced neuronal activation compared with depressed animals, indicating an active coping process. Further experiments are needed to examine whether deferential plastic changes in the synapses onto mPFC neurons in response to stress are critical in determining an animal's susceptibility or resilience to behavioral depression.

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

Genetic Deficiency, Glutamatergic Hypofunction, and Schizophrenia

In this project, we are studying the genetic causes of glutamatergic hypofunction, a pathological process believed to contribute to the etiology of schizophrenia. We first focus on *ErbB4*, a gene that has been linked to both schizophrenia and bipolar disorder. We choose to study brain circuits that have the highest levels of ErbB4 expression.

The thalamic reticular nucleus (RT) is critical in processing and filtering sensory stimuli. It is thought to have an important role in attention, and impairment in RT function has been implicated in psychiatric disorders such as schizophrenia. RT neurons, which are exclusively GABAergic, receive glutamatergic inputs from the cortex and thalamus and send projections to the thalamus where they modulate the activity of thalamic relay neurons. Interestingly, RT neurons are highly enriched in ErbB4. Previous studies indicate that ErbB4 controls glutamatergic synapse maturation and plasticity. On the basis of these findings, we hypothesize that ErbB4 controls the development and function of glutamatergic synapses in RT neurons, thereby controlling the normal function of the RT–thalamic circuitry in attention. To manipulate ErbB4 function specifically in the RT, we took advantage of the observation that somatostatin (SOM) expression perfectly matches that of *ErbB4* in the RT, but not in other brain areas including cortex and hippocampus. By crossing the SOM-Cre line with the *ErbB4*^lox/lox animals, we have generated SOM_ErbB4$^{-/-}$ mice in which the *ErbB4* gene is selectively ablated in SOM-positive neurons. We are currently examining the potential synaptic changes in RT neurons in these animals and testing the role of the RT-thalamic circuitry in a behavioral task designed to specifically test attentional modulation of behavior.

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Hong Goo Chae

INTEGRATIVE SYSTEMS NEUROBIOLOGY

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               P. Grange   S. Mukhopadhyay   D. Prevzner
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Our basic research philosophy is to combine theoretical, computational, and experimental approaches for the study of complex biological systems, with a particular focus on neurobiological questions. Efforts in our group fall into three main areas: informatics, theory, and experimental work.

Our neuroinformatics research involves the application of analytical tools to large volumes of neurobiological data, as well as the development of informatics infrastructures for data and knowledge integration. Our study in the area of theoretical engineering applies theories developed in human-engineered systems to study the theoretical principles underlying biological systems. The final area of our research is experimental and has previously consisted of collaborative studies in multiple species including *Drosophila*, zebra finch, macaque monkey, and human infants. We are now undertaking a major in-house experimental project to generate the first brainwide mesoscale connectivity map in the mouse.

Continuing in our laboratory in 2010 are Pascal Grange (postdoctoral fellow), Jamie Jones (laboratory technician), Jayant Kulkarni (postdoctoral fellow), Sandra Michelsen (administrator), Vadim Pinskiy (graduate student), and Caizhi Wu (research associate). Swagatam Mukhopadhyay (postdoctoral fellow), David Prevzner (laboratory technician), Alex Tolpygo (laboratory technician), Gregor Havkin (informatics manager), Osama El Demerdash (analyst), Wen Huang (graduate student), Joshua Novy (laboratory technician), Andrew Weber (laboratory technician), Zhen Gong (graduate student), Linzie Wood (project assistant), and Thomas Pologruto (consultant) joined us this past year.

We also have close collaborative ties with multiple research groups at CSHL and other institutions, which currently include Dr. Nicholas D. Schiff at the Weill Medical School of Cornell University, where Dr. Mitra is an adjunct associate professor; a consortium of zebra finch researchers; an integrative analysis of memory formation in *Drosophila* with Josh Dubnau at CSHL; the Brain Architecture Project, with collaborators at multiple institutions, notably Harvey Karten (University of California, San Diego),

Kathy Rockland (Massachusetts Institute of Technology), and Michael Hawrylycz (Allen Institute of Brain Research); and a collaboration with Gregory Hannon at CSHL on improving next-generation sequencing technologies.

Mouse Brain Architecture Project

V. Pinskiy, A. Tolpygo, C. Wu, P. Grange, J. Jones, D. Prevzner, A. Weber, J. Novy, G. Havkin, O. El Demerdash

Brain function is determined by its circuitry, but very little is known about how the mammalian brain is connected. We have set out to address this problem in the Mouse Brain Architecture Project, in which we are comprehensively determining the patterns of neural connectivity between brain regions.

We have obtained two National Institutes of Health (NIH) grants (a Transformative-R01 and an RC1 Challenge Grant) that are running concurrently to produce the connectivity map. The RC1 grant is to produce a connectivity draft for wild-type C57BL/6J mice and to align these results to the Allen Brain Atlas. The initial draft of a connectivity atlas will be available by fall or winter of 2011. The T-RO1 grant will be used to increase the sample size and coverage and to compare connectivity maps of schizophrenia and autism mouse models to a reference connectivity map in the C57 mouse brain. These data and the data analysis tools will be released on the Mouse Brain Architecture project website.

This project is now in full production phase. The pipeline to process tissue from tracer-injected mice through to image analysis is fully functional and all equipment has been integrated into a Laboratory Information Management System (LIMS). This was developed in-house and serves as an electronic lab notebook, facilitates quality control procedures, and is also the metadata repository that will be finally published on the web.

Our plan involves grouping injections into major brain systems (i.e., cortical areas, basal ganglia, thalamus, hypothalamus, etc.). Each injection site yields information about the other regions of the brain projecting to and

from the injected region. We are incorporating specific advice from anatomists who specialize in these individual areas, in terms of injection strategies and interpretation of results. The initial 200 injection sites consist of a set covering the cortex in a defined grid. CTB (cholera toxin B), BDA (biotin dextran amine), fluorescent-modified rabies viruses, and AAV (adeno-associated virus) will be the initial tracers. We have already injected more than 400 production animals at 100 different sites.

For these experiments, a computer-guided apparatus for stereotactic injections into the mouse brain has been developed. A laser scanner of the mouse skull, assembled by Vadim Pinskiy and Thomas Pologruto, gives rise to a profile of the surface of the skull of the live animal. A reference atlas of the mouse skull was provided by Mark Henkelman (Mouse Imaging Center, University of Toronto). A registration algorithm developed by Pascal Grange allows us to align the skull and to work out its variation in size relative to the reference. The injection is guided accordingly. The method was presented at the Society for Neuroscience meeting in November 2010.

Other improvements to the pipeline include development of an embedding system that consistently orients the brains for sectioning and allows two brains to be sectioned simultaneously. We have also developed a Giemsa counterstain for the BDA and CTB tracers. This increases the signal/noise ratio for the diaminobenzidine (DAB) staining and subsequent registration. A set of Nissl-stained sections for each brain will be used to capture the cytoarchitecture. All of these improvements to the pipeline increase both the throughput rate and the proper segmentation of individual brains and registration to a common atlas space. The 20-μM brain sections are imaged using an automated microscope and new and updated software has been written to aid with data cropping, transfer, and storage. This will be incorporated into the LIMS and used for quality control of the tissue samples and data analysis. Other software components, currently under development, will take the stacks of digitized microscopy images and perform segmentation and three-dimensional reconstruction to reconstitute the three-dimensional brain. This brain volume is then registered with the Allen Reference Atlas (ARA) (Fig. 1).

Although the total amount of data generated by the project is expected to be large (terabytes to petabytes), we expect that the principal challenges will be in terms of software to enable suitable compression schemes to permit effective transmission through the Internet (which remains a low-bandwidth medium), as well as suitable visualization and search tools. The Allen Institute has extensive experience in this domain, and Dr.

Mitra has established a collaborative relationship with Dr. Mike Hawrylycz at the Allen Institute.

The preliminary data will be made available to the research community via an informatics platform (along with basic informatics tools to query, organize, visualize, and manage the data). We have implemented software to transform proprietary image data into readable and web-compatible formats and are beginning to assemble the hardware and software infrastructure for sharing image data with collaborators and the outside world. We anticipate that the data set produced will be important for analysis of many kinds, and we expect to be actively engaged in new analyses even beyond the 5-year grant period. The availability of public data sets and open-source duplicable pipeline technologies will potentially impact the entire neuroscience community.

We are additionally developing a novel technique for assaying long-range connectivity in the human brain and are pursuing the aims of mining and integrating information about brain connectivity from the research literature using computational linguistics techniques. Finally, this project has also benefited from a summer course that Dr. Mitra cofounded at CSHL in the beginning of June 2010. Through this project, Cold Spring Harbor Laboratory has become a leading center for filling the critical knowledge gap urged by Dr. Crick, and championed by Jim Watson.

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

Analysis of Gene Expression in the Mouse Brain

P. Grange

We are determining networks of spatial coexpression in the brain, of addiction-related genes. An initial list of more than 400 genes related to addiction was compiled from on-line databases. For each pair of genes in the list, the spatial overlap between gene expression patterns across the brain was computed. The resulting coexpression networks of genes can be rendered graphically—genes with similar expression energies appear as clustered nodes. These graphs have been made available online to the research community. This project was supported the NIDA grant 1R21DA027644-01, *Co-expression networks of addiction-related genes.*

We also developed numerical methods to rank genes and sets of genes as markers of brain regions. Localization scores for individual genes are available online (www.addiction.brainarchitecture.org, which also shows
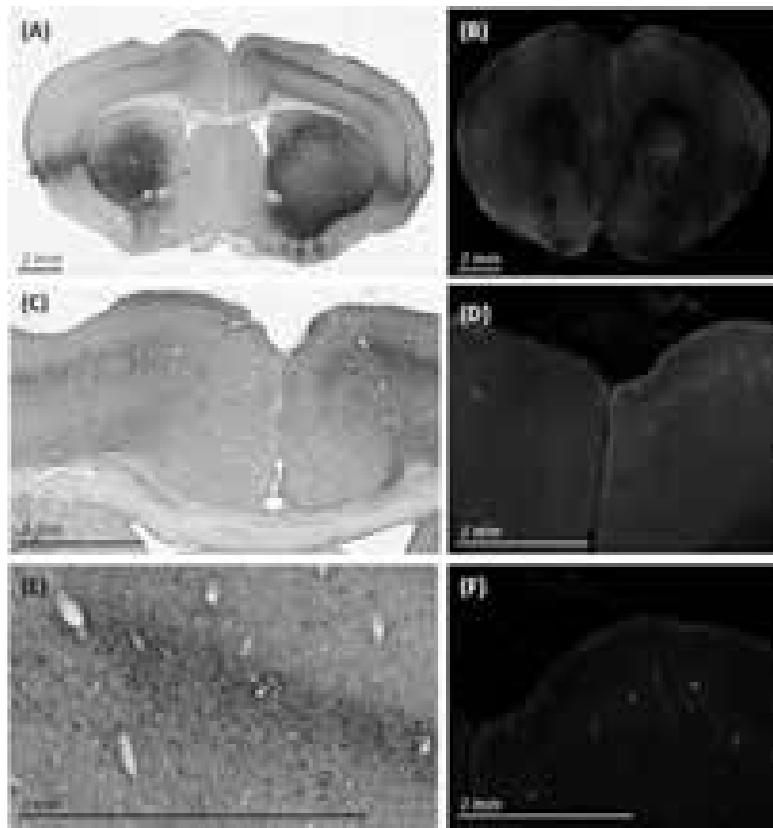
Figure 1. Examples of processed materials from the pipeline. Cholera toxin, subunit B (*A,C,E*) and retrograde mutant rabies virus (*B,D,F*) were injected into the caudoputamen of 56-d-old C57BL/6 mice (two separate animals; tracer injection placed in right caudate close to the labeled sections). (*A,B*) Full coronal sections containing tracer label, (*C,D*) presence of retrograde labeled neurons in both ipsilateral and contralateral cortex, consistent with known bilateral projections from cortical pyramidal cells to caudoputamen, (*E,F*) individual labeled neurons.

results of the coexpression study described above). Optimal sets of genes with coefficients determined by linear-algebraic methods can have much higher scores than individual genes. These results were presented for 12 major brain regions at the Society for Neuroscience meeting in November 2010. Results for finer brain parcellations are being prepared for publication. In collaboration with Claudio Mello (Oregon Health and Science University), we are determining whether mouse-brain-region markers show a similar pattern of gene expression in the zebra finch brain. Such comparisons will provide quantitative tests of hypotheses regarding conservation of brain regions during evolution.

We are also determining the evolutionary ages of genes cataloged in the Allen Institute mouse brain atlas, with the goal of discovering whether there are correlations between the spatial pattern of gene expression in the brain and their evolutionary ages. We intend to provide evolutionary age as an added layer of information to brain-spe-

cific genes, with the hope of understanding whether major anatomical regions of the brain have characteristic signatures in the profile of evolutionary ages of their representational genes. For this purpose, two separate data sets of orthologs from published work are being used—the OMA data set and the Ensembl data set. The former is a comprehensive list of all orthologs found among ~1000 species, including ~100 eukaryotes. By grouping genes into sets of first appearance of homologs in major phylogenetic taxa, we are able to order genes in the set being analyzed by evolutionary age. Integrating this information with the gene expression data is in progress.

Next-Generation Sequencing: Data Analysis
S. Mukhopadhyay

We are working on analytic methods for improving genomic analyses, using a next-generation sequencing plat-

form (single-molecule real-time sequencing, Pacific Biosystems). In collaboration with the McCombie lab at CSHL, the objectives of this project are to investigate error profiles in sequencing results and to improve base-calling algorithms by modeling the physical processes involved in polymerase kinetics and signal from the fluorescent-tagged (phospholinked) nucleotide incorporation. The short-term goal includes developing algorithms for direct detection of DNA modifications by utilizing their signatures on polymerase kinetics. We are in the process of developing new tools for the various sequencing methods used in the platform, such as Strobe sequencing and Circular Consensus sequencing. Our long-term goal is to use the single-molecule signature of polymerase kinetics to refine our understanding of polymerase motor itself.

Multimedia Signal Processing for Quantative Phenotyping in Autism Spectrum Disorders

Z. Havkin

We have been studying phenotypes of autism spectrum disorders (ASDs) with support from the Simons Foundation Autism Research Initiative (SFARI). The work has focused on the application of multimedia signal processing (MMSP) techniques to audiovisual recordings of ASD patients made during the administration of a standardized diagnostic instrument (the Autism Diagnostic Observation Schedule). The ultimate goal of this research is to objectively determine early-age ASD phenotypes, as it has been previously shown that early diagnosis and intervention leads to substantially improved outcomes.

Our key clinical collaborators are Woodfords Family Services in Maine and the Autism & Communication Disorders Center at the University of Michigan. We will be applying our MMSP techniques to their longitudinal studies which include ASD patients, non-ASD patients, and typically developing peers.

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Alexander Tolpygo

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

P. Osten   Y. Bao   R. Palaniswamy   K.U. Venkataraju
          L. Kadiri   N. Takada   D. Xu
          Y. Kim   J. Taranda   L. Zhang

What causes autism and schizophrenia? It is well established that both disorders are highly heritable, and today, geneticists are uncovering the relevant susceptibility genes and loci. The wealth of genetic data, however, does not guarantee rapid progress in understanding the disease mechanisms. In fact, the susceptibility genes comprise a heterogeneous group that includes genes regulating transcription, protein degradation, synaptic signaling, and cell adhesion. What do these genes have in common? How can we best progress from identifying genes to testing therapeutic strategies?

We have developed a high-throughput pipeline of methods that can be used to systematically search for changes in brain functions in mouse models carrying genetic mutations linked to autism and schizophrenia. Our current work aims at identifying disrupted brain circuits that lie downstream from *autism* genes. Once such circuit deficits are known, we and others can focus on determining the underlying cellular and molecular mechanisms. Importantly, the same methods that we use to discover disrupted circuits in the mouse brain can be also used to screen drugs that eliminate the abnormality and restore normal functions. Our approach thus uniquely links basic and translational research, promising rapid progress from mechanistic studies to testing therapeutic strategies.

Sectioning-Based Serial Two-Photon Microscopy

A novel imaging technique, which we termed sectioning-based serial two-photon (SSTP) microscopy, is a key part of our experimental approach (Fig. 1). The SSTP microscope, developed in a collaboration with Tissue-Vision, Inc. (Cambridge), works as follows: (1) A top view of a fixed mouse brain is imaged as a mosaic of 20x objective fields of view ("tiles"), (2) a built-in vibratome cuts off the imaged top region, and (3) the cy-
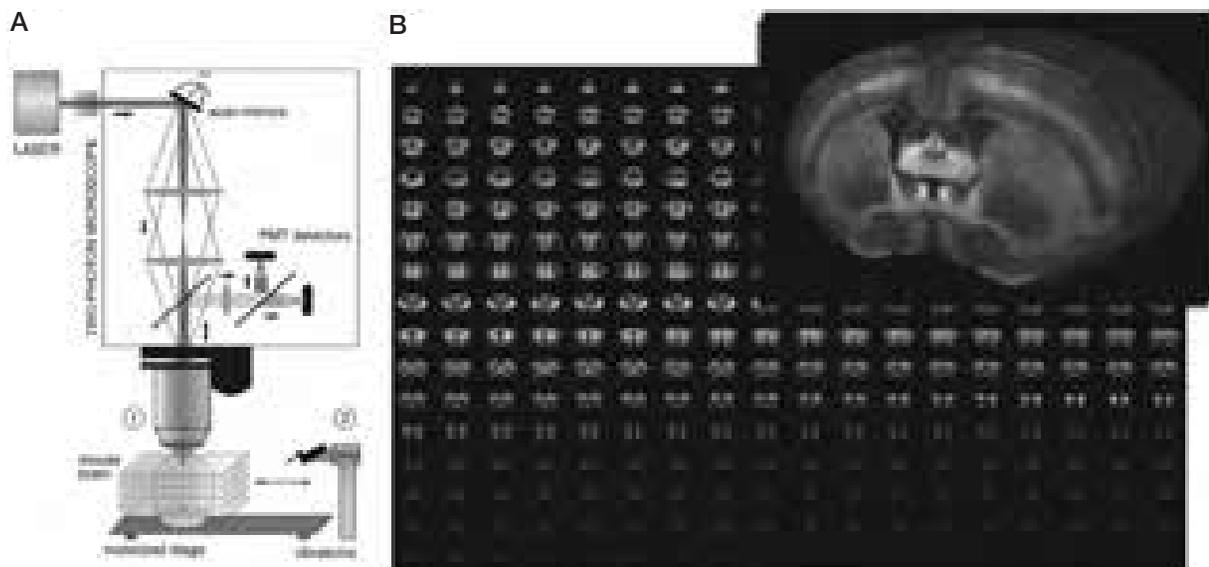
Figure 1. SSTP microscopy. (*A*) Microscope scheme. The x-y-z stage moves the brain under the objective, so that the top view is imaged as a mosaic. The stage also moves the brain to the vibratome for sectioning. (*B*) An example of a GFP (green fluorescent protein) mouse brain imaged in 280 coronal sections.
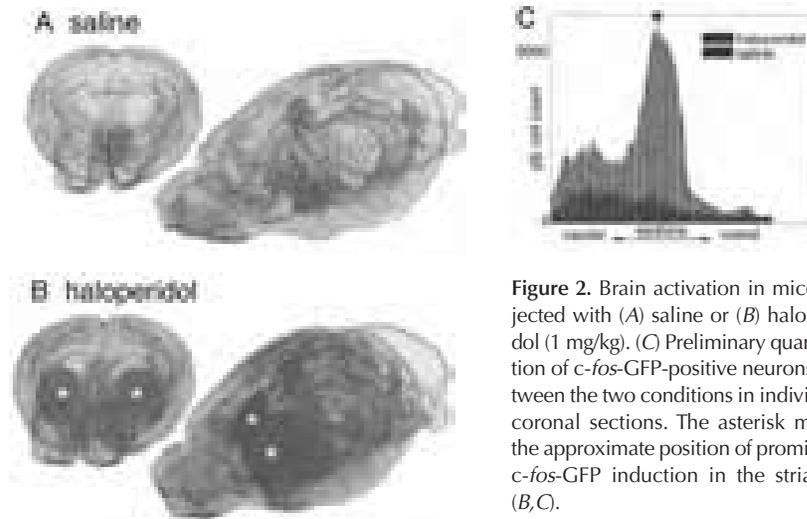
Figure 2. Brain activation in mice injected with (*A*) saline or (*B*) haloperidol (1 mg/kg). (*C*) Preliminary quantitation of c-*fos*-GFP-positive neurons between the two conditions in individual coronal sections. The asterisk marks the approximate position of prominent c-*fos*-GFP induction in the striatum (*B,C*).

cles of mosaic imaging and sectioning are repeated. Once all data are collected, image-processing and brain-morphing algorithms are used to assemble the three-dimensional brain volume for data analysis (Fig. 1).

Transgenic "Indicator" Mice

To study mouse brain functions by SSTP microscopy, we use transgenic indicator mice expressing GFP as a reporter of neural activation, such as c-*fos*-GFP mice expressing GFP from the promoter of the activity-regulated immediate early gene (IEG) c-*fos.* These mice enable visualization of brain activation at cellular resolution in response to behavioral tasks or applications of drugs, such as the antipsychotic drug haloperidol (Fig. 2).

The use of SSTP microscopy to screen brain circuits in genetic mouse models of autism involves (1) breeding of the selected mouse models with the indicator c-*fos*-GFP mice and (2) analysis of c-*fos*-GFP activation in the mouse offspring. We have initiated work on five mouse models of autism: 16p11.2 deletion and duplication mice generated by our collaborator Alea Mills at CSHL and previously published Neuroligin R451C, Neuroligin 4 knockout, and CNTNAP2/Caspr2 knockout mice. We currently focus on two questions. We are testing a hypothesis that autism susceptibility genes cause an imbalance in brain excitation and inhibition, which leads to higher propensity for seizures and contributes to disruptions of brain circuits relevant to autism manifestation. In a parallel set of experiments,

we are testing a hypothesis that the functions of brain circuits involved in mediating social interactions are disrupted downstream from autism candidate genes. The aims of both experiments are (1) to find differences in brain functions between mutant and wild-type littermates and (2) to compare these differences between different mutants in order to identify common disruptions downstream from multiple autism genes.

Anatomical Tracing and Electrophysiology

Our current work focuses on identification of brain areas and circuits disrupted in five genetic mouse models of autism. Once such regions are found, our next step is to use electrophysiology and anatomical mapping to study in detail the cellular mechanisms underlying the circuit deficits. Electrophysiological experiments are done in vitro in acute brain sections and in vivo by whole-cell recording in anesthetized mice. Anatomical mapping is done using traditional tracers, such as virus-vector-based GFP expression for anterograde tracing and cholera toxin B for retrograde tracing (Fig. 3).

Summary

We hope that our work will provide better understanding of neural circuit disruptions underlying autism and schizophrenia and lead to therapeutic developments in the future.
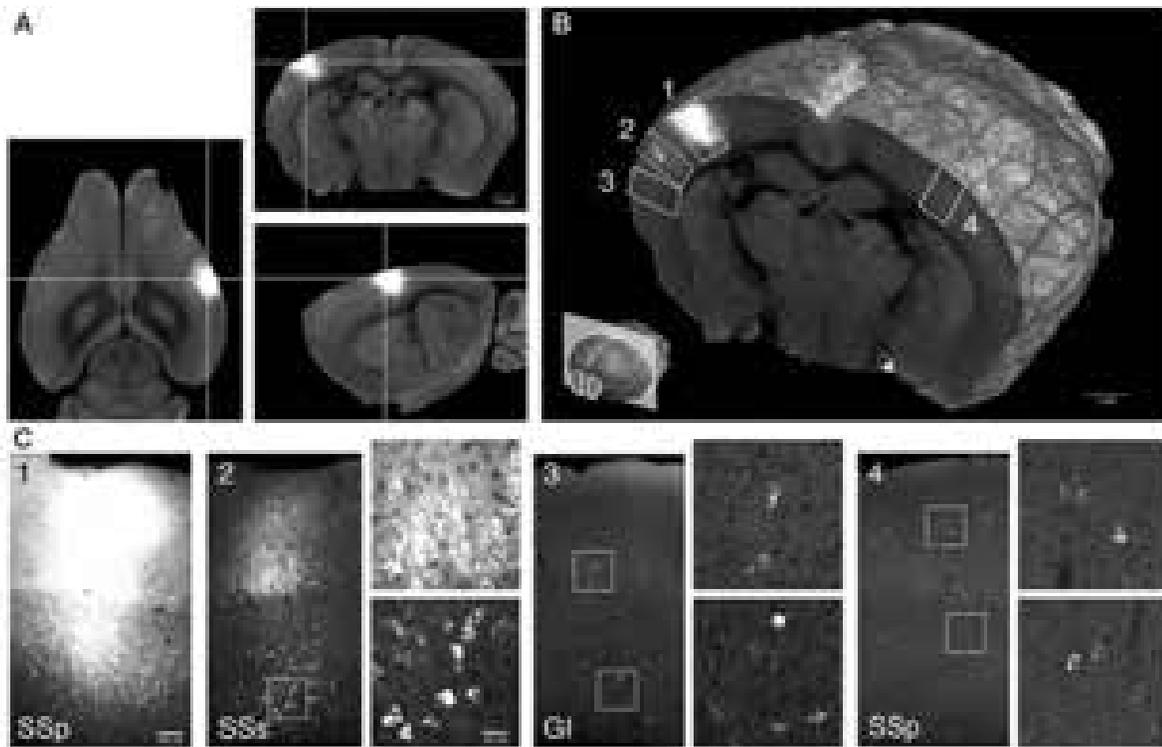
Figure 3. Retrograde tracing with Alexa-conjugated CTB. (*A*) Overview of the injection area. (*B*) Injection area of the barrel cortex (1) and retrogradely connected cortical areas (2–4). (*C*) Enlarged views of the marked areas.

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

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

The major focus of our laboratory is to identify genetic causes of mental illness using a successful approach that we pioneered: high-resolution analysis of rare copy-number variants (CNVs) in the human genome. We have developed an experimental design that allows us to identify rare mutations that confer high risk for disease. Our approach is to screen the genomes of patients with schizophrenia and bipolar disorder to identify deletions and duplications of the DNA that disrupt genes. These mutations are subsequently tested for association with disease in families and in large samples of patients and healthy individuals. This approach is based in part on the findings of our previous genetic studies of autism spectrum disorders (ASDs), which established an important role for rare spontaneously occurring CNVs in the etiology of ASDs. With support from the Stanley Foundation, we have now successfully applied this approach to studies of schizophrenia and bipolar disorder, and our work has already had a significant impact on the field. Our findings, coupled with studies by other groups, have shown that rare CNVs have an important role in the genetics of psychiatric disorders. Furthermore, we have shown that a substantial number of the risk variants detected in the DNA of patients are not inherited from a mother or father but instead occur by spontaneous mutation. Most individual CNVs that have been definitively identified to date increase risk of mental illness by ≥10-fold, strongly suggesting that these mutations have a causal role.

Analysis of Copy-Number Variation in Schizophrenia

V. Vacic, S. McCarthy

Studies by our group and others have demonstrated that individually rare structural variants contribute to risk of schizophrenia (SZ) and other neurocognitive disorders. Multiple studies have now shown that the mutational burden of rare structural variants is significantly greater in patients with SZ than in healthy controls. In addition, specific loci have been identified where structural muta-

tions are significantly associated with SZ. For example, large deletions at 1q21.1, 15q13.3, and 22q11.2 have been shown to confer substantial risk of SZ. A recent study by our group has found that microduplications of 16p11.2 are significantly associated with SZ (Fig. 1, Table 1; see McCarthy et al., *Nat Genet 41:* 123 [2009]). The SZ-associated regions described here confer significant risk. For instance, the observed odds ratios for the microduplication of 16p11.2 range from 8.3 to 25.8.

These findings suggest that rare structural variants have a role in the etiology of SZ. Furthermore, evidence suggests that little of the overall contribution of rare CNVs to SZ can be explained by the handful of loci described above (Sebat et al., *Trends Genet 25:* 528 [2009]). We hypothesize that genetic risk of SZ consists in part of rare variants, and these risk alleles involve many different genes. Analysis of structural variation in larger samples is required to definitively identify novel CNV risk factors.

In an effort to identify novel CNV risk factors in SZ, we have recently carried out a two-stage study of copy number variation, using a primary sample of 742 cases and 856 controls scanned in the Nimblegen HD2 platform, and using as a replication data set CNV calls made from the GAIN and MGS studies of SZ and the International Schizophrenia Consortium (ISC). The replication sample consisted of 7488 cases and 6689 controls, bringing the combined sample size to 8230 cases and 7545 controls.
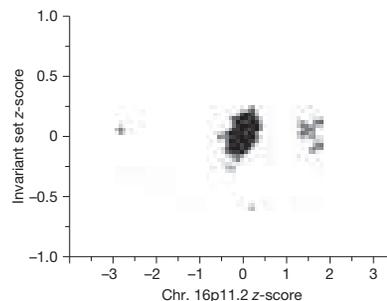
Figure 1. Detection of 16p11.2 microduplications by MeZOD.

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

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

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

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

Investigating the Role of Rare CNVs in Bipolar Disorder

D. Malhotra

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

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

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

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

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

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

Analysis of Structural Variation in the 1000 Genomes Project

S. Yoon, K. Ye

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

our CNV calls and to better define the breakpoints of variants identified in this study.

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

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NEURAL CIRCUITRY FOR SOCIAL COMMUNICATION

S. Shea   H. Demmer   D. Eckmeier
         J. Dos Santos   R. Hu

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

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

There are three broad areas of research in the lab. One concerns olfactory communication and memories

for familiar individuals. Another direction is an examination of vocal (auditory) communication between mother mice and their pups. Our final avenue of study involves recording from neurons in awake, behaving animals to reveal the neural encoding of social cues during a live encounter with another mouse.

Noradrenaline and Memories for Familiar Individuals

H. Demmer, D. Eckmeier, J. Dos Santos

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

The ability to induce ethologically relevant memories under anesthesia opens up exciting possibilities for observing the synaptic mechanisms underpinning such memories using advanced techniques that are currently impossible in behaving animals. For example, we are beginning to use a variety of imaging approaches in genet-

ically modified mice during memory formation. These experiments will allow us to separately visualize olfactory bulb input and output as well as wide-scale neuronal populations to ascertain how and where NA-dependent plasticity is coordinated and interacts among neuronal populations. We are also beginning to use high-resolution electrophysiological techniques, targeted to specific cell types in order to build a circuit picture of how different olfactory bulb cell types adapt their firing to result in long-term changes to circuit output.

How are olfactory memories for individuals stored mechanistically among the specific synaptic connections of the various neuronal types in the olfactory bulb? To answer this question, this year in our lab, Dr. Heike Demmer developed techniques for making technically challenging targeted recordings from a specific type of inhibitory neuron (granule cells) whose function remains mysterious. She then made these recordings during the induction of NA-dependent plasticity to examine how they contribute to memories. The data suggest that these cells not only participate in storing olfactory memories, but do so in surprising and complex ways. Although we anticipated that the granule cells might increase their activity to suppress responses to remembered odors, we found the opposite was true (Fig. 1). One interpretation is that NA input uncouples granule cells' synaptic output from their action potential firing. This unique property could be an important factor in maintaining memory specificity.

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

Vocal Communication between Mothers and Pups
R. Hu

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

One form of vocalization that is actually reasonably well understood is the ultrasonic distress vocalization (USV). Young mice prior to vision and full mobility will occasionally become separated from the nest. This is stressful for them and they will therefore call out to their mother with a very-high-frequency peep. New
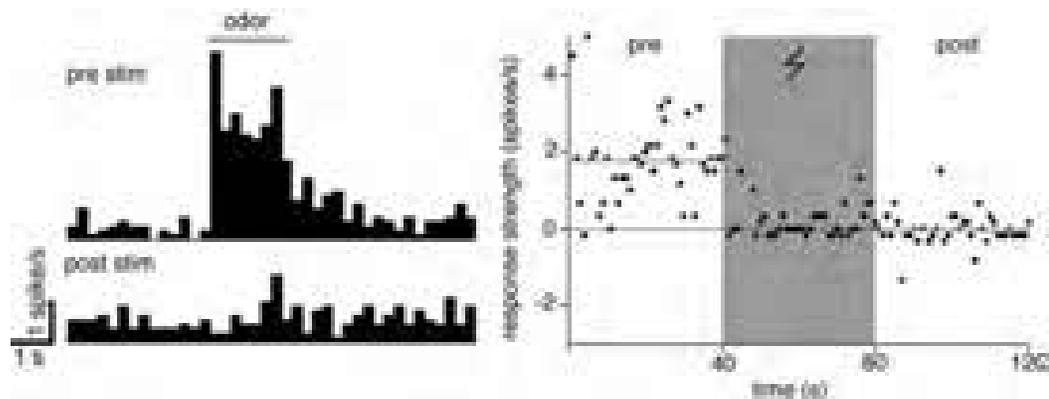
Figure 1. Granule cell interneurons of the main olfactory bulb show long-term suppression of odor responses after locus coeruleus (LC) stimulation. This figure depicts the effects on a granule cell's odor responses of electrical LC stimulation (which releases NA) paired to that odor. (*Left panel*) Two PSTHs showing the pre- and poststimulation response of the granule cell to the odor. (*Right panel*) A plot of response strength (spikes/s above baseline) for each of 120 consecutive trials of odor presentation with the shaded region denoting the period of LC stimulation pairing.

mothers develop sensitivity to these cries and respond by moving toward their source (phonotaxis) to retrieve the pup. Mothers will also approach a speaker emitting playback of synthetic calls, providing a simple assay for their perception of manipulated calls. Such experiments suggest that there are sharp limits to the types of sounds that will elicit phonotaxis, possibly implying a neural selectivity mechanism that creates a perceptual boundary between pup distress cries and other sounds and vocalizations. Moreover, nulliparous females who have never given birth do not show approach responses to pups or their calls, suggesting that the underlying neural responses may also differ.

To begin to search for such neural correlates of maternal behavior, Ruilong Hu, a summer URP student, made recordings from the auditory cortex of mothers and naïve nulliparous females and assessed the response to a series of synthetic pup calls. He found that neurons in the auditory cortex of mothers were much more sharply tuned to pup-call frequencies as compared to the neurons of naïve females (Fig. 2). These data imply that somehow maternal experience rewires the brain to more optimally discriminate biologically important stimuli. How is this neural selectivity constructed at the synaptic level? We have hypothesized that the answer critically involves intracortical inhibitory processing. Therefore, we are using optogenetic techniques to in-activate inhibitory inputs to ultrasound-sensitive neurons to assess their necessity for proper auditory processing.

Neural Activity during Social Encounters
D. Eckmeier

We are building a setup for recording individual neurons during social encounters and other behavioral assays involving the perception of social and nonsocial information. There are two broad related goals to this approach. The first goal is to examine the encoding of social information such as body odors and vocalizations in primary sensory structures of awake animals. We hypothesize that activity in response to these signals may be labile to associative learning, attention, and arousal which we may be able to manipulate in the context of social encounters. The second goal is to record from neurons in deep-brain neuromodulatory centers during these encounters as well. Neurons that release noradrenaline and dopamine are likely responsive to social signals and may modulate encoding of sensory data and associative plasticity. Understanding the context-dependent activity patterns of these neurotransmitters is therefore critical to developing models for how they affect behavior.
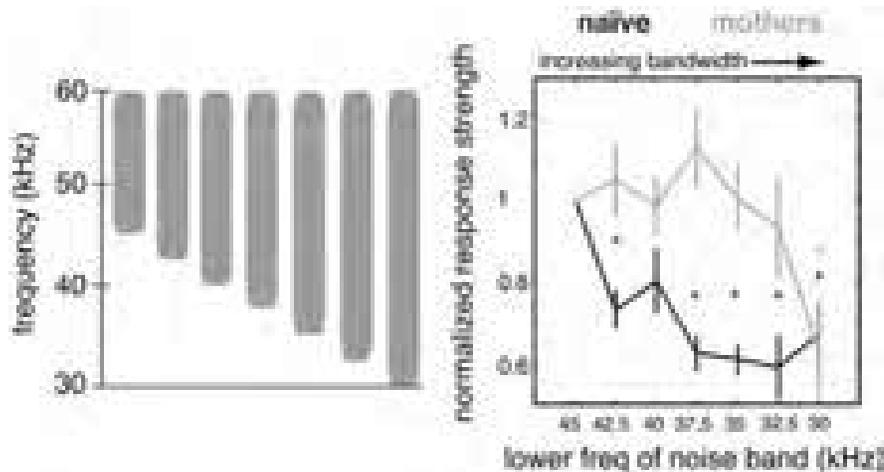
Figure 2. Auditory cortical neurons in mothers show greater spectral selectivity for USV frequencies than neurons in naïve females. (*Left panel*) A schematic representation of the bandpass noise stimuli that were randomly presented to anesthetized mothers and naïve females during recordings of auditory cortical neurons. (*Right panel*) Neurophysiological results. As more low-frequency content was added to the stimulus, responses in mothers dropped off precipitously, demonstrating that these neurons are more selective for USV content. All responses were normalized to the response to the narrowest band stimulus.

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Heike Demmer

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

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

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

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

Functional Imaging of Population Coding in Olfaction: Neural Activity to Perception

R. Campbell, K. Honegger

A central goal in systems neuroscience is to understand the relationship between neural activity and sensory perception. How different do two response patterns have to be in order to be perceived as distinct? This difference can be considered a unit of the neural code: the smallest difference in activity that the animal can perceive. We have addressed this question in the olfactory system of *Drosophila*, focusing on a brain area known as the MB, which is essential for learned olfactory discrimination. Using imaging techniques to track more than half the population of MB neurons, we address this question with a completeness not currently feasible in mammalian systems.

We compared behavioral measures of olfactory discrimination with neural activity patterns monitored using calcium imaging. Flies were trained to form a Pavlovian association with one odor and then to choose between the trained odor and a different odor in a T maze. To track neural activity, we targeted expression of the calcium sensor, GCaMP 3.0, specifically to MB neurons using genetic tools available in *Drosophila*. We used two-photon imaging to record activity patterns, incorporating a piezoelectric z-motor to image the entire MB in a three-dimensional volume during a single odor presentation. This approach enabled us to routinely monitor neural activity of most MB neurons. By measuring odor responses on a trial-by-trial basis, we could accurately quantify response variability. This enabled us to construct an algorithm to classify odors based on the patterns of active neurons, taking into account the noise inherent in neuronal responses.

MB odor responses were sparse, consistent with electrophysiological recordings. Different monomolecular odors could be readily distinguished from one another based on activity patterns, even when odors evoked exceptionally similar patterns of activity in the population of olfactory receptor neurons. Behavioral tests showed a similar level of discriminability. We then tested a series of progressively similar binary blends of odor, which enabled us to construct a psychometric curve characterizing discriminability as a function of stimulus similarity. We established a corresponding neurometric curve describing our ability to classify odors based on those activity patterns. There was a close correspondence between the neurometric and psychometric curves, indicating that

we can interpret the patterns of MB activity in naive flies in a way that accurately predicts how finely they can learn to discriminate odors. Overall, our results show that small differences in input are nevertheless easily distinguished in the MB population, a transformation that likely underlies accurate information storage.

Robustness of Sparse Coding in the *Drosophila* Mushroom Body
K. Honegger, R. Campbell

Theoretical work suggests that sparse representations are useful for learning and memory because they enable synaptic changes to modify representation of one stimulus without interfering with another. However, an important underlying assumption is that these sparse representations are robust to natural variations in the stimulus. There are two broad challenges to maintaining a sparse representation: variations in stimulus intensity and variations in stimulus complexity. We have examined how the sparse, specific MB responses are maintained in the face of these challenges.

We examined robustness to stimulus intensity by linearly varying odor concentration across two orders of magnitude. We found that in general, responses are extremely similar across different concentrations, a useful feature that would enable odor recognition independent of intensity. Interestingly, there is a form of plasticity that sets this invariance: When the same odor is presented in a series of pulses of steadily increasing concentration, the MB response appears to be completely concentration invariant. When pulses are steadily decreasing, however, response strength is proportional to odor concentration. This suggests that when traveling up a concentration gradient, the MB only conveys information about odor identity, perhaps to confirm that the odor is still present, whereas decreasing odor intensity should be readily detectable by neurons downstream from the MB.

To evaluate the effects of stimulus complexity, we tested responses to multicomponent odors, including naturally occurring smells. Several considerations suggest that natural odors may evoke responses in the MB fundamentally different from those produced by monomolecular odorants. In other systems such as vision, it is the stimuli that have meaning to the animal that evoke strong responses in neurons at the deeper layers. Studying deeper olfactory areas with monomolecular odorants the animal has never before encountered may have led to an impoverished view of MB response properties. Additionally, our observations that individual

Kenyon cells (KCs) integrate input from several different projection neurons (PNs) (see below) raise the possibility that many KCs respond selectively to odor blends, such as natural odors. In contrast to these expectations, we find that natural odors evoke responses that are comparably sparse and selective to responses to monomolecular odorants.

Additionally, imaging allows us to determine whether odor responses are in some way spatially clustered, with pockets of dense activity. In earlier layers of the olfactory system, there is a clear spatial organization of responses; however, in the MB, no obvious structure exists and responses appear to be distributed randomly. We also ruled out the possibility that MB neurons are spatially arranged according to their odor-tuning properties. Altogether, these results are consistent with the notion that the MB is a purely associative area: Natural stimuli that have intrinsic meaning do not elicit qualitatively distinct responses from monomolecular stimuli nor is there any obvious spatial segregation based on odor response preferences that would suggest olfactory information is channeled into distinct anatomical pathways.

Integration Properties of MB Neurons
E. Gruntman

The nervous system has to represent a large number of different stimuli using a relatively small number of neurons. At the sensory layer, combinatorial coding by large ensembles of neurons enables a small number of cells to represent a much larger number of different stimuli. In deeper layers, sparse representations by highly stimulus-selective neurons can ensure that similar stimuli evoke markedly distinct patterns of activity, a feature thought to be important for accurate memory formation. The olfactory system in *Drosophila melanogaster* displays these fundamental characteristics: The olfactory receptor neurons represent odors combinatorially, and they are maintained as functional units by projecting to form synapses in distinct glomeruli within the second layer of the system, the antennal lobe. At the third layer, a learning and memory area termed the MB, neurons known as KCs are highly stimulus specific and odor responses are relatively sparse in this population.

One hypothesis for how KCs achieve their high odor specificity is that they integrate across several different input channels and require the combined activity of those inputs in order to spike. Projection neurons from the second layer of the system relay information from distinct glomeruli to the KCs; however, it is yet unknown whether each individual KC receives informa-

tion from the same glomerulus, thus acting as an amplifier, or from different glomeruli, acting as an integrator.

We expressed the calcium sensor GCaMP3.0 in single KCs and used two-photon imaging to monitor the olfactory responses of individual dendritic sites in a living fly. This enabled us to construct an odor-tuning curve for each dendritic input site. By comparing tuning curves for different sites, we directly tested whether KCs integrate combinations of different inputs and whether the integration is a prerequisite for a spiking response. Our results suggest that some KC types integrate information whereas others mainly amplify. We also found that KC spiking requires simultaneous activation of most of the input units. By integrating across different input channels, KCs in effect read the combinatorial code of the earlier layers.

Decoding Sparse Representations

T. Hige

Sparse representations are useful for learning and memory, but how do downstream neurons integrate this information? Ultimately, the information in layers with sparse representations has to be converted into a behavioral response. How does this process occur and how do neurons downstream from a sparsely responding brain area integrate that information?

In *Drosophila,* this process appears to occur immediately after the MB, as the 2500-Mb neurons converge onto an estimated 50 output neurons. Individual members of this output population can be green fluorescent protein (GFP) labeled using the genetic tools available in *Drosophila*, making this an excellent system to determine the basic principles of information processing downstream from sparse representations. Using GFP-targeted intracellular recordings, we have characterized the odor responses of one of these MB output neurons. In contrast to the highly odor-specific tuning of MB neurons, this output cell responds to a broad array of different odors. These functional properties are reflected in the cell's anatomy: It has very extensive dendritic processes within the output lobes of the MB, suggesting it receives highly convergent input from many MB neurons. Although the neuron responds to almost all tested odors, it nevertheless responds distinctly to different odors, suggesting that this neuron is capable of carrying odor-specific information. However, the odor-tuning properties of this neuron vary among individual flies—the same neuron has very different tuning curve shapes in different animals. One explanation for this variability is that it reflects synaptic plasticity that operates on a developmental timescale. Our working hypothesis is that the tuning curves of the MB output neurons are a product of the prior olfactory experience of the individual fly. We are currently testing this by examining the role of olfactory experience in shaping the response properties of these neurons.

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NEURAL CIRCUITRY OF UNDERLYING NORMAL AND ABNORMAL PROCESSING IN THE CORTEX

A. Zador K. Borges   T. Hromadka   H. Oviedo   A. Reid   H. Zhan
               G. Cao   S. Jaramillo   H. Oyibo   Q. Xiong   P. Znamenskiy
               S. Chattopadhyay S. Koh   S. Peikon   Y. Yang

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

Sequencing the Connectome

S. Chattopadhyay, G. Cao, H. Oyibo, S. Peikon, H. Zhan
[in collaboration with J. Dubnau, L. Enquist, Princeton University]

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

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

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

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

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

Disruption of Auditory Cortical Circuits by Autism Candidate Genes

T. Hromadka, Q. Xiong

Autism is a highly heritable disorder thought to arise through disruption of neural circuits. Many candidate

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

Role of Interneurons in Auditory Cortex Function

K. Borges, A. Reid

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

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

Processing of Spatial Information in the Auditory Cortex

S. Koh

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

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

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

Temporal Expectation Modulates Neuronal Responses in the Auditory Cortex

S. Jaramillo

When a stimulus occurs at a predictable instant in time, anticipation of the stimulus improves the speed and accuracy with which it is detected. We have developed a two-alternative choice task paradigm in freely moving rats to study the neural mechanisms underlying this phenomenon in the auditory system. Behavioral measurements confirm that valid expectations improved both reaction times and detection thresholds. We are also using tetrodes to record responses from single neurons in the auditory cortex of rats performing the task. Responsive neurons often showed an increased evoked response to tones immediately preceding the expected moment of appearance of the target when compared against responses to the same tones occurring long before the expected target. In addition, grouping behavioral trials according to the subject's reaction time reveals correlations between the strength of the neuronal responses and performance (Jaramillo and Zador 2011).

Mapping of Auditory Cortex Circuitry Using Laser-Scanning Photo Stimulation

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

It is widely assumed that the organization of the sensory cortex can be described by a "canonical" circuit. According to this view, sensory input from the thalamus arrives at cortical layer 4, propagates to layer 2/3, and then descends to layer 5 before exiting a brain region. However, until recently, it has been technically difficult to test this hypothesis directly. We are applying a new approach—laser-scanning photostimulation—to map the circuitry within the rodent auditory cortex. Using this approach, we can directly compare the circuitry within the auditory cortex with that of other sensory cortices, such as the better-studied barrel cortex. Our results indicate that although the auditory cortex is organized according to many of the same general principles, the detailed structure appears to be quite different (Oviedo et al. 2010).

Using Cortical Timing Information to Guide Behavior

Y. Yang, G. Otazu, M. DeWeese

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

We have therefore adopted a direct approach to probe the precision with which cortical timing information can be used to guide behavior in the rat (Yang et al., *Nat Neurosci 11:* 1262 [2008]). To bypass subcortical auditory pathways, we stimulate the primary auditory cortex directly, using transient biphasic current trains delivered via chronically implanted intracortical microelectrodes. The behavioral paradigm we use is a two-alternative choice task in which stimulus 1 consists of the simultaneous stimulation of two intracortical sites (A and B), and stimulus 2 consists of sequential stimulation of the two sites separated by a brief interval dt(A–dT–B). After the subjects are trained to perform to criterion (1–2 weeks) with the initial long interstimulus interval (dT > 50 msec) for stimulus 2, we probe the subjects' psychophysical threshold by reducing dT, until the subjects can no longer distinguish between the two stimuli. Our experiments indicate that the cortex can make use of information on a timescale as short as 3 msec (Yang et al., *Nat Neurosci 11:* 1262 [2008]).

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong   J. Beshel   Y.-C. Shuai
             B. Lu   C. Xu

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

Trace Conditioning in *Drosophila*
Y.-C. Shuai

Trace conditioning is valued as a simple experimental model to assess how brains associate events that are discrete in time. Organisms as simple as the fruit fly are able to acquire trace conditioning by learning to avoid an odorant that precedes an electric shock punishment by many seconds. We observed that this type of learning is supported by mechanisms remarkably divergent from the well-characterized simultaneous conditioning, in which presentation of the odorant and punishment overlap. First, the rutabaga-encoded adenyl cyclase, which is central to simultaneous conditioning and is thought to act as a coincidence detector, turns out to be dispensable in trace conditioning. Second, inhibition of the Rac-forgetting mechanism does not affect acquisition of simultaneous conditioning. Remarkably, this same manipulation enhances trace conditioning.

Food-Seeking Neuron in *Drosophila*
J. Beshel

For all motile organisms, survival depends on accurately identifying and localizing appropriate food sources. Odors are one of the most important sensory cues animals use to track, evaluate, and select among available foods. Fruit flies are no exception, using olfactory cues to navigate their environment and make determinations regarding the relative merits of following such a cue to its source. We identified the neuropeptide Y homolog *Drosophila* neuropeptide F (dNPF) as critical in mediating innate and satiety state-appropriate approach behaviors to olfactory cues from likely food sources. We monitored single dNPF-positive neurons and showed that these neurons respond to olfactory stimuli with increases in intracellular calcium. In vivo odor-evoked activity profiles are larger in response to the category of natural food odors than to other complex odorants, synthetic monomolecular odorants, or monomolecular components of natural food odors. Moreover, these increases are satiety-state-dependent, with hungry flies exhibiting larger levels of activity as compared to satiated flies. Remarkably, the level of dNPF neuronal activation exactly parallels the degree of odor-associated food seeking in hungry and satiated flies and furthermore suggests coding of preference as opposed to simple binary attraction-aversion. Additionally, silencing dNPF neurons eradicates food seeking in hungry flies, whereas activating these neurons makes satiated flies behave as though hungry. Together, these results suggest that single neurons can encode the significance of an encountered stimulus, adjusting its comparative worth based on internal need. Such neurons, as we demonstrate, ultimately may predict behavior with astonishing specificity.

NF1 and Long-Term Memory in *Drosophila*
C. Xu

Neurofibromatosis type 1 (NF1) is a single gene disorder that affects about one in every 3500 people in the

population. The gene involved, named neurofibromin, encodes a large 25-kD protein and is highly conserved throughout the animal kingdom. In addition to tumor predisposition in various somatic tissues, NF1 patients also show learning difficulties and cognitive defects. *Drosophila melanogaster* is an established learning and memory model, and its NF1-coding sequence shares ~75% similarity with that of human NF1. For these two reasons, *Drosophila* is an attractive model for studying how NF1 affects cognitive function. Our lab has previously reported that flies lacking NF1 show impaired immediate memory. After repetitive training, NF1 mutants can eventually perform as well as wild type, but they are unable to sustain the same level of long-term memory (LTM) as wild-type animals. This indicates that NF1 is involved in LTM. The stages and biochemical pathways involved in turning the immediate memory into LTM, however, are complicated and less well understood. We are currently conducting behavioral experiments on NF1 mutant animals to uncover which stages are NF1 dependent.

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Jennifer Beshel

PLANT GENETICS

Uncovering the molecular processes governing how plants grow and develop is of fundamental biological interest and importance, with vital implications both for agriculture and human health. Plant genetics has had a major role in the advance of all areas of research at CSHL almost since the Laboratory's inception, epitomized by the work of Nobel laureate Barbara McClintock, who discovered transposable genetic elements in her work on maize in the 1940s. Plant geneticists at CSHL have been among the leaders of efforts to sequence the first plant genomes and continue to be at the center of sequencing and genome-annotation projects involving a host of cereal crops that feed the planet's growing population. CSHL plant geneticists also have been pioneers in the study of RNA interference, stem cell research, and, most recently, efforts to spur the development of next-generation biofuels and related alternative energy sources.

David Jackson and colleagues study genes and signals that regulate plant growth and architecture. They are investigating a unique way in which plant cells communicate, by transporting regulatory proteins via small channels called plasmodesmata. These channels, which direct the flow of nutrients and signals through growing tissues, are regulated during development. The team discovered a gene, *CCT8*, that controls the transport of a transcription factor, KNOTTED1, between cells in the plant stem cell niche, or meristem. KNOTTED1 is critical for stem cell maintenance, and studies of the *CCT8* gene indicate that movement of KNOTTED1 between cells is required for this function. The lab also continues to identify other genes that control plant architecture through effects on stem cell maintenance and identity. A recent example is the discovery of a subunit of a heterotrimeric G protein that is conserved throughout animals and plants; their studies indicate that this gene controls stem cell proliferation. A second example is the identification of a gene, *GRASSY TILLERS1*, that appears to have been instrumental in the domestication of the corn plant, a process that led to the development of agriculture ~10,000 years ago. The lab has also started to characterize system-wide networks of gene expression in inflorescence development, using "next-generation" profiling methods. Jackson has also undertaken to develop a collection of maize lines that can drive expression of any reporter or experimental gene in any tissue type. These tools, of great interest to maize researchers, are being made available to the broader scientific community, enabling experiments never before possible in crop plants.

Zachary Lippman's research focuses on identifying genes that cause tomato plants to produce their flowers in an elegant repetitive zigzag arrangement on a branching structure called an inflorescence. Proceeding from a base of knowledge that they have built regarding a universal growth habit represented by the tomato, called sympodial growth, Lippman's lab is addressing the fundamental question of how plants are able to stop making leaves and start making flowers. Of particular interest is how these "reproductive phase transitions" have contributed to the evolution of diverse inflorescence branching patterns in the tomato's larger Solananaceae family, which includes plants that make just one flower in each inflorescence as well as plants whose inflorescences produce dozens of branches and hundreds of flowers. Using classical and modern genetic techniques, Lippman is dissecting the gene networks that are responsible for the variation in inflorescence branching found in nature. He hopes to leverage these discoveries to improve crop yields in several Solanaceous plants such as pepper, eggplant, and potato. Already, a collaboration between Lippman's group and scientists at Hebrew University has identified a gene called *florigen* that is responsible for making flowers in plants and can be altered using simple genetic tricks that push hybrid tomato plants to increase their yield by as much as 60%. Lippman plans to extend this finding to corn through a collaboration with David Jackson's group.

Epigenetic mechanisms of gene regulation—chemical and conformational changes to DNA and the chromatin that bundles it—have important impact on genome organization and inheritance and on cell fate. These mechanisms are conserved in eukaryotes and provide an additional layer of

information superimposed on the genetic code. Robert Martienssen, a pioneer in the study of epigenetics, investigates mechanisms involved in gene regulation and stem cell fate in yeast and model plants including *Arabidopsis* and maize. He and colleagues have shed light on a phenomenon called position-effect variegation, caused by inactivation of a gene positioned near densely packed chromosomal material called heterochromatin. They have found that heterochromatin is programmed by small RNA molecules arising from repeating genetic sequences. Recently, Martienssen and colleagues described a remarkable process by which "companion cells" to sperm in plant pollen grains provide them with instructions that protect sperm DNA from transposon damage. With collaborators in Mexico, Martienssen this year coaxed *Arabidopsis*, a flowering plant, to reproduce asexually in a process related to apomixis. The key was shutting down the Argonaute 9 protein, which tricked an ovule into manufacturing multiple gametes, rather than one. These gametes carried the full complement of genetic material for the next generation. This is an important step in a long-time goal of plant breeding: generating clonal offspring to perpetuate hybrid vigor. Martienssen's group also continues to work on problems related to the creation of plant-based biofuels.

The growing tips of plants, called meristems, contain a population of stem cells that serve as a persistent source of daughter cells from which new organs arise. They also produce signals important for the determination and patterning of lateral organs. Marja Timmermans and colleagues are using a genomic approach to study genes active in the meristem. They have also used mutational analyses to unravel the mechanism that suppresses stem cell fate during organ development. They have shown that this process requires a highly conserved epigenetic mechanism. In particular, they have found that the chromatin-remodeling factor HIRA, through interaction with specific DNA-binding proteins, mediates the recruitment of Polycomb repressive complexes to stem cell factors, an action that stably represses their expression in differentiating organs. The Timmermans lab has also identified regulatory mechanisms that allow for the precise spatial accumulation of developmentally important small RNAs in plants. This work has revealed that polarity in leaves is established via opposing gradients of mobile small RNAs that act as morphogen-like signals. 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. Mathematical modeling suggests that such opposing small RNA gradients might serve to generate robustness during development.

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

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             K. Chen   N. Hariharan   L. Lefevre H. Szilagyi F. Yang
             S. DeBlasio U. Hernandez   L. Panda   J. Wang   T. Zadrozny
             A. Eveland   B.I. Je

Our research aims to identify genes, signals, and pathways that regulate plant growth and development. All organisms develop by carefully controlling the flow of information ("signals") that passes between cells and tissues. We are particularly interested in discovering these signals and finding out how they are transmitted and how they function. As an example, we have identified genes that control how plant cells communicate with each other through small channels, called plasmodesmata. These channels were described in plants more than 100 years ago, but their central importance and how they are regulated is just beginning to emerge. Plasmodesmata are critical for plant growth, because they allow the passage of nutrients and signals through growing tissues. In the past year, we elucidated an important mechanism by which transport through these channels is controlled, via a chaperonin gene that regulates the specific transport of a transcription factor, KNOTTED1. This factor is a homeodomain protein that is expressed in the plant stem cells and is necessary to keep these cells in a pluripotent state. We found that KN1 function depends on chaperonin activity, highlighting the importance of cell-to-cell trafficking in plant stem cell function.

We also continue to identify maize genes with novel roles in shoot development and plant architecture. We have recently identified genes that control branching, stem cell proliferation, and leaf growth. One of these, *compact plant2*, encodes a subunit of a heterotrimeric G protein that has previously not been linked with stem cell proliferation in plants. Studies of CT2 have, for the first time, linked the action of a plant hormone, giberellin, with the control of stem cell proliferation. Finally, we continue to develop a collection of "fluorescent-protein"-tagged maize lines that are an essential resource for all maize researchers. This is the first collection of its kind and promises to enhance maize genetics research through characterization of developmental gene pathways and identification of maize promoters that can be used in crop improvement.

Characterization of a Dominant Phyllotaxy Mutant, *Abphyl2*, in Maize

F. Yang, V. Llaca, H. Sakai, DuPont Crop Genetics, Delaware; Robyn Johnston, Cornell University, New York

Plant morphology and diversity are largely dependent on phyllotaxy, which is the geometric arrangement of new leaf primordia formed by the shoot apical meristem (SAM). Auxin and its polar transport through PINFORMED1 (PIN1) proteins are crucial for controlling phyllotactic patterns. Recently, our studies on *ABPH1* in maize have shown that cytokinin hormone signaling, as well as its cross-talk with auxin, have an important role in this process. *abphyl1* (*abph1*) mutations change maize phyllotaxy from alternate to decussate. Here, a similar phyllotaxy mutant, *Abph2*, is described. *Abph2* is dominant and originated from a Chinese inbred line; it has a decussate leaf pattern that becomes visible at leaf ~4–5 stage. Map-based cloning done in three genetic backgrounds has mapped *Abph2* in a region of ~20 kb on chromosome 7, containing five predicted genes in the reference B73 genome. However, direct sequencing of the open reading frames (ORFs) of these five genes, as well as their transcript level analysis by reverse transcriptase–polymerase chain reaction (RT-PCR), did not give any obvious clues as to the identity of the *Abph2* gene. Next, a bacterial artificial chromosome (BAC) library generated from the *Abph2* mutant was screened using probes located within the 20-kb mapping interval and identified six positive clones. One of them fully covers the 20-kb mapping interval. We are currently analyzing the sequence of this BAC to obtain clues into the molecular basis of the *Abph2* mutation. Meanwhile, putative knockout lines of the dominant *Abph2* mutation were screened by ethylmethanesulfonate (EMS) mutagenesis. These putative knockouts will be used to confirm the gene, once a candidate has been identified. In addition, introgression lines are being

generated to investigate the influence of different genetic backgrounds on *Abph2* phenotypes. Among three introgression lines in A619, B73, and W23 backgrounds, respectively, *Abph2* phenotypes show a dramatic enhancement in A619, characterized by splitting of the SAM into two to three distinct shoots. In contrast, B73 and W23 appear to weaken the *Abph2* phenotypes, since the decussate leaf pattern becomes visible much later. Analysis of additional introgression lines are in progress. Finally, informed by the fact that auxin patterning and PIN1 maxima in the leaf initiation site are diminished in *abph1*, we are currently analyzing auxin distribution in *Abph2* meristems, using PIN1-YFP and DR5-RFP as markers. Preliminary analysis revealed that the expression of PIN1 in the *Abph2* SAM appears to be decreased, suggesting a common mechanism by which these mutants control phyllotaxy. More detailed analysis to confirm these observations is under way.

Regulation of the Cell-to-Cell Trafficking of the Transcription Factor KNOTTED1: Identification of Chaperonins and Additional Factors through Genetic Screens

X.M. Xu, J. Wang, L. Panda, N. Hariharan

Cell-to-cell communication has critical roles in specifying cell fate and coordinating development in all multicellular organisms. A special paradigm for such communication in plants is the selective trafficking of informational macromolecules, e.g., transcription factors and small RNAs through plasmodesmata (PDs), channels that traverse the cell wall and connect all plant cells. In addition to cell-fate specification, PDs are also involved in viral movement, transport of metabolites, and cell-to-cell spread of RNA interference (RNAi), which points to their fundamental importance in coordinating plant defense, metabolism, and development. Despite the discovery of PD more than 100 years ago, and our increasing recognition of their functional significance, the underlying components and mechanisms of PD trafficking remain poorly understood. Hence, we are taking an unbiased genetic strategy to dissect these molecular components and mechanisms, using a transgenic reporter system.

The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PDs, and its trafficking has been suggested to be important for its function in shoot stem cell maintenance. A gain-of-function trafficking assay in *Arabidopsis* was developed to demonstrate that the carboxy-terminal region of KN1 (KN1^C), which contains the homeodomain, is necessary and sufficient for KN1 trafficking in vivo. The trafficking assay relies on complementation of leaf hair (trichome) development in hairless *glabra1* (*gl1*) mutants. GL1, a MYB transcription factor, is required in the epidermis for trichome initiation and acts cell autonomously. However, expression of a fusion between GL1 and KN1^C in cells underlying the epidermis can rescue trichomes in a *gl1* mutant background, because the fusion protein can traffic through PDs into the epidermal cells. Thus, this system provides a simple and tractable model to understand how proteins traffic cell to cell. Factors critical for KN1 trafficking can be uncovered through isolating mutants defective in trichome rescue. Through screening ~2500 M2 plants, several mutants have been obtained which all show an attenuated KN1 trafficking.

Through combining traditional mapping and illumina-based high-throughput sequencing, one such mutant was identified as a chaperonin gene. Using artificial microRNA knockdowns, we found that additional chaperonin subunits also function during KN1 trafficking, suggesting that the whole chaperonin complex is required for trafficking. Physical association between the chaperonins and KN1 also strengthens the idea that the chaperonins are directly involved in KN1 trafficking. Genetic interaction data provide evidence that the endogenous function of KN1-related genes in *Arabidopsis* also requires chaperonin, and chaperonins are coexpressed with KN1-related genes in the SAM (Fig. 1), clearly supporting the functional relevance of chaperonin-mediated trafficking through PDs. Furthermore, tissue-specific complementation assays point to a role for chaperonins during the posttranslocational refolding process. In addition, chaperonins also appear to be essential for the PD trafficking of non-cell-autonomous proteins other than KN1, suggesting chaperonin dependency is a general mechanism for protein trafficking. Together, our data highlight the importance of conformational changes for PD trafficking and identify a chaperone that promotes protein trafficking through PDs.

Meanwhile, we also took a transgenic approach to address the in vivo significance of KN1 cell-to-cell trafficking. For the ease of transformation, we worked with *Arabidopsis* and used the *Arabidopsis* homolog of KN1, SHOOTMERISTEMLESS (STM), as our model protein. Like KN1, STM has also been shown to move between cells, and mutations in *STM* cause a defect in
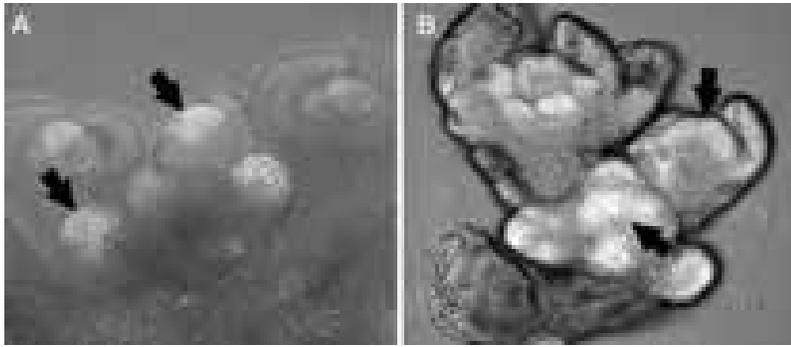
Figure 1. Expression of the chaperonin gene (CCT8-GFP, *B*) overlaps with that of STM-YFP in the shoot apical meristem (*A*); expression is shown as white areas and is arrowed.

meristem maintenance. Through attaching a nuclear localization signal (NLS) to STM, we have developed a modified version of STM with reduced mobility. In the *stm* mutant background, we expressed this less mobile form of STM in its native expression domain. By asking if the less-mobile STM can complement *stm* mutants, we can tell whether mobility is essential for its function. Preliminary data showed that the less-mobile STM complements the mutant less well, when compared to the nonmodified STM, suggesting that mobility is important for proper STM function. Efforts are under way to address at which developmental stage, and where in the meristem, STM movement is required. In summary, our analysis has furthered our understanding of the developmental regulation and mechanisms of selective cell-to-cell trafficking.

Illuminating Maize Biology: Using Fluorescent Proteins to Study the Mechanisms Controlling Maize Inflorescence Development

S. DeBlasio, A. Goldshmidt, T. Zadrozny, J. Wang [in collaboration with A. Chan, J. Craig Venter Institute; A. Sylvester, University of Wyoming]

Like animals, the formation and patterning of reproductive structures (inflorescences) in maize depend on hormonally induced changes in gene expression that occur within meristematic stem cells in response to cues from the environment. These stem cells and their daughter cells undergo several important identity transitions to produce the male tassel and the female ear inflorescences. First, a highly organized group of stem cells called SAM develops within the embryo and starts generating the vegetative organs of the plant, including the leaves. Later in development, the SAM transitions into the inflorescence meristem, which in turn pro-

duces several files of intermediate meristems along its flank, which will produce the floral structures. How these floral organs develop under normal and stress conditions has a major effect on agronomic processes including crop yield and nutrient content. Therefore, studying the underlying mechanisms controlling meristem maintenance and differentiation will ultimately uncover guiding information for crop improvement. One of the ways in which our lab is studying these processes is through the use of fluorescent protein (FP) technology.

When fused to a protein, FPs make the attached, normally invisible protein visible and easy to track. Because of their relatively small size, FP fusions rarely interfere with native protein targeting, function, and trafficking. Thus, FP technology is a powerful tool that can be used to noninvasively mark protein expression and localization to study various subcellular processes, live and in vivo. To date, we have generated more than 90 stable, natively expressed, maize FP fusion lines that highlight most subcellular compartments and allow us to study hormone signaling, cytoskeletal behavior, vesicle trafficking, and other important developmental and physiological processes. For example, a modified FP synthetic reporter for the plant hormone auxin has provided an easy way to visualize the changes in auxin signaling that occur during inflorescence development. This reporter introgressed into the classical *ramosa* mutants (in which spikelet pair meristems lose determinacy and form branches) has revealed distinct differences in how auxin is distributed within the mutant meristems, even before they deviate morphologically from normal development (Fig. 2). We are also using these lines to immunoprecipitate tagged proteins and isolate additional components of these pathways. In the case of FP-tagged transcriptional regulators, chromatin immunoprecipitation (ChIP) is also being used to identify putative DNA-binding sites and/or to confirm gene targets that have
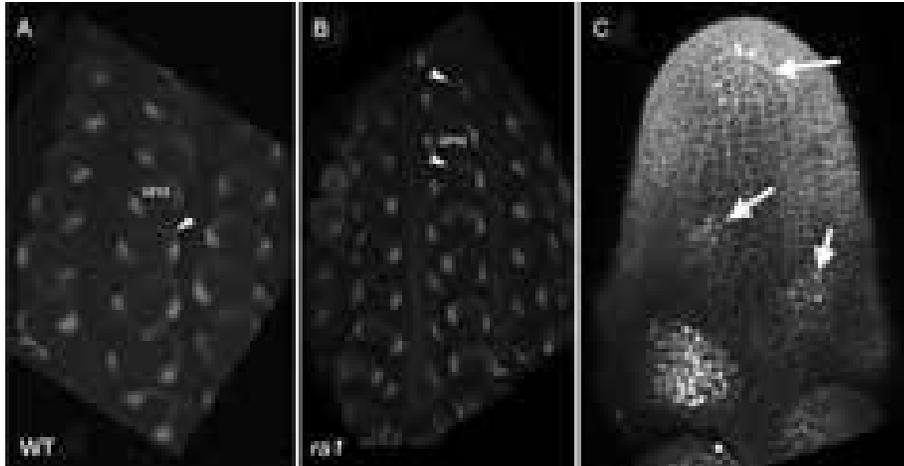
Figure 2. Maize fluorescent protein reporters. Expression of the synthetic auxin reporter DR5::RFP.ER in (*A*) wild-type (WT) and (*B*) *ramosa1* (*ra1*) maize ear primorida. In wild-type spikelet pair meristems (SPM), an auxin maximum forms discretely along the edge of the meristem, whereas in *ramosa1*, an additional auxin maximum can be detected in the central region of the SPM, prior to the manifestation of the mutant phenotype (white arrows). (*C*) Expression of p*WUSCHEL*::4XNLS-RFP reports the stem cell niches in ear meristems (arrows).

been detected by transcriptional profiling of the respective mutant.

Currently, we are working on optimizing the LhG4 two-component *trans*-activation expression system for use in maize. This system relies on the transcription factor LhG4 being expressed by a cell or tissue-specific promoter, to drive the expression of a reporter in *trans*, via the pOp-binding sites. The reporter can be any gene, such as GUS, FPs, an FP fusion protein, a gene of interest for tissue-specific expression, a silencing construct for tissue-specific knockdowns, or a gene encoding a toxin for ablation experiments. Our goal is to produce an array of cell- and tissue-specific driver lines, which we will use to analyze the changes that occur within meristems during differentiation and development. From our previous work creating natively expressed, FP-tagged protein fusion lines, we have already identified several meristem-specific promoters, such as p*ZMM16*, p*ABPHYL1,* and p*WUSCHEL* (Fig. 2), which we will initially use to test the reliability of the system. Driver lines will include new maize optimized FP color variants, as well as a tagged ribosomal protein (RPL18) for tissue-specific profiling by immunoprecipitation of translating mRNAs. Data on the characterization of all of the FP transgenic lines we have created, including confocal micrographs and movies, are publicly available on http://maize.jcvi.org/cellgenomics/index.shtml. The website also contains a community submission form to request your favorite maize gene or regulatory element to be tagged.

Role of the *fasciated ear2* Gene in Maize Seed Production and Crop Improvement

P. Bommert, Y.K. Lee [in collaboration with M. Komatsu, H. Sakai, Dupont Crop Genetics]

The cob structure of maize is unique among the grasses, and it is derived from the distichous (two-rowed) inflorescence of its wild-type ancestor teosinte, through more than 9000 years of domestication. Teosinte seeds are encased in hard shells and the teosinte inflorescence is characterized by only two alternating rows of seed, whereas maize ears display hundreds of exposed kernels arranged in up to 24 rows. This morphological change is considered an important factor for the massive increase in yield associated with the domestication of maize.

Seed row number is dependent on the number of spikelet pair meristems, and we were able to show that this number in turn is directly dependent on the size of the inflorescence meristem. Following genetic approaches, we were able to identify several genes controlling the size of the inflorescence meristems in maize (see also section on fasciated mutants). One of them is the *fasciated ear2* (*fea2*) gene, which, when mutated leads to plants with strongly enlarged inflorescence meristems and more than 40 rows of seeds.

To address whether allelic variation in *fea2* has an impact on seed row number, we performed a quantitative trait locus (QTL) analysis using 250 lines of the intermated B73-Mo17 (IBM) recombinant inbred popula-

tion, in three replicates. The mean row number of B73 was ~18, whereas Mo17 develops ~11 rows. The mean row number in the IBM families was ~14, with a range from ~10 to 20. We found the most significant seed row number QTL to be located on chromosome 4, overlapping with the *fea2* locus, suggesting that allelic variation in *fea2* could regulate row number.

To substantiate these findings, we searched for hypomorphic EMS-induced alleles, via TILLING. We were able to isolate four new alleles of *fea2*, all predicted to be weak. Analysis of seed row number in three of them showed a statistically significant increase. Subsequent genotyping confirmed a significant correlation between row number increase and presence of the newly identified, weak *fea2* allele, providing further evidence that allelic variation at the *fea2* locus can lead to changes in seed row number. Field trials to assess the impact of increased seed rows on maize yield are currently in progress.

The Regulation of Meristem Size in Maize

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A. Eveland, J. Kendall [in collaboration with M. Komatsu,
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All plant organs derive from populations of stem cells called meristems. These stem cells have two purposes: to divide in order to replace themselves and to give rise to daughter cells, which will differentiate into lateral organs or stems. Consequently, plant meristems must precisely control the size of the stem cell niche via a network of positive and negative feedback signals. A loss of function in a negative regulator of stem cell fate can result in an enlarged or fasciated meristem phenotype and a dramatic alteration in the morphology of the maize ear and tassel.

Maize is an excellent genetic model system, because of a large collection of developmental mutants and a sequenced reference genome. Our lab has undertaken a forward genetic approach to identify key regulators of stem cell homeostasis and meristem size. Two previously cloned mutants, *fasciated ear2* and *thick-tassel dwarf1*, encode orthologs of the *Arabidopsis thaliana* genes *CLAVATA1* and *CLAVATA2*, indicating the well-known *CLAVATA-WUSCHEL* regulatory feedback loop is conserved from dicots to monocots. However, little else is known about the control of this important developmental process in maize. Here, we describe our progress in identifying additional genes contributing to stem cell niche homeostasis.

ct2 is a classical mutant of maize that exhibits a fasciated inflorescence phenotype as well as semidwarfism. *ct2* was identified by positional cloning and was found to encode the α-subunit of a heterotrimeric GTPase. The protein was localized by a fluorescent protein translational fusion and was expressed strongly in meristems. Several experiments demonstrated that *ct2* is deficient in gibberrellic acid (GA) signaling, a plant hormone with no previous connection to meristem size control. We are currently using both genetic and proteomic approaches to determine how *CT2* interacts with established pathways for regulating meristem size.

Another fasciated ear mutant that we recently cloned is *fea3*. *fea3* is predicted to be similar to previously described stem cell receptor genes, and sequence analysis indicates that it might encode a membrane-bound receptor. The *fea3* mutant possesses several favorable characteristics relevant to crop improvement. First, plant height does not seem to be affected as in most fasciated ear mutants. Second, although mutant tassels have a higher spikelet density than wild type, the overall size of the tassel is not reduced. This could lead to increased pollen shed, a desirable trait for hybrid crop production. Future work will include characterizing the spatial expression of *fea3* and profiling the transcriptome to understand which pathways with which it interacts.

fea1905 is another semidwarfed mutant, with massively fasciated ears and tassels. We mapped the mutant to an ~2.7-Mb region on chromosome 6, which contains ~30 genes. We are using targeted resequencing by genomic hybrid capture of the interval to find the causative mutation. Probes were designed for Agilent 244K arrays that tiled gene models in the mapping interval, and hybridization was carried out according to protocols from Emily Hodges (Hannon lab, CSHL). Preliminary results suggest that the amount of DNA captured is limited and not enriched for the target region. We are also beginning to use mRNA-Seq to search for mutations in transcripts arising from within the interval. A complementary approach is to create double mutants to assemble genetic pathways. We have analyzed double mutants between *fea1905* and *ramosa1* and *ramosa2*, two mutants that display increased inflorescence branching. The *ramosa* mutants appear to be epistatic to *fea1905*, indicating that they likely act downstream. This represents a previously unknown connection between meristem size control and meristem determinacy. Future work will include molecular cloning of the gene, expression analysis, and transcriptome profiling.

In addition, we are in the process of mapping ~50 fasciated ear mutants from nontargeted EMS mutagenesis screens. Our current approach involves creating F_2 mapping populations and rough mapping by bulked segregant analysis. We take advantage of the precise quantitative genotyping capability of the Sequenom MassArray system to look for areas of the genome that are linked to the phenotype. We have determined rough positions for several of these mutants and are proceeding with fine mapping and molecular cloning.

Resolving Networks Controlling Axillary Meristem Determinacy

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

A rising challenge in developmental biology is elucidating the mechanisms by which genes are regulated in space and time, and how their actions form networks that modulate differentiation and development. We use the maize inflorescence as our model to study how branching patterns are determined at the molecular level and the genetic interactions responsible for inflorescence architecture. Branching in grass inflorescences is determined by the developmental fate of highly organized stem cell populations called axillary meristems. These meristems can either maintain continual growth of indeterminate branches or terminate in determinate structures such as flowers. Actions of genes that regulate meristem determinacy, as well as the genetic interactions between them, are responsible for the wide variation in architectures among inflorescences throughout the plant kingdom. The genetic control of branching in maize, especially in ears where kernels are borne, has clear relevance to grain yield and harvesting ability. In addition, its unique inflorescence architecture, characterized by flowers borne on spikelet pairs, is shared exclusively among the Andropogoneae tribe, which includes important energy crops such as sorghum, switchgrass, and sugarcane. Loss-of-function mutations in the *RAMOSA* (*RA*) genes cause a branched phenotype, due to derepression of branching in spikelet pair meristems (SPMs). We make use of these branching mutants as well as Illumina's high-throughput sequencing technology to identify transcriptional signatures related to a gene network controlling inflorescence architecture.

Primary objectives of this work are to establish a framework for studying developmental networks that control branching and to use both experimental and computational approaches to generate and address testable hypotheses. Initially, this framework consists of large-scale expression data sets and known genetic interactions. Candidate genes identified as putative regulators of meristem determinacy will be tested as to whether they interact with *RA* genes and for their position in a network controlling SPM determinacy. This year, we published results from an analysis of sequence-based expression profiles in maize inflorescences, which identified genes that were differentially expressed between wild-type and *ramosa3* mutants. We identified strand-specific expression signatures and classes of differentially expressed genes having putative functions in SPM determinacy. Work is currently under way to analyze loss-of-function mutations in these candidate loci, as well as crosses to the *ramosa* mutants to study potential genetic interactions.

We have also generated mRNA-Seq libraries at various stages of development from ears and tassels as well as from loss-of-function *ra* mutants. A pipeline for analysis of these data sets was developed and includes methods for data processing, mapping short reads to the maize reference genome, and statistical analyses to identify differentially expressed genes and/or those sharing similar expression patterns. We leveraged a set of high-confidence maize gene models and comparative functional information from other model plant species to classify transcription factor (TF) family members, based on their specific expression signatures in time and space. We used a clustering approach to group coexpressed genes during ear and tassel development. As predicted by their colocalized expression in ear primordia, *RA1* and *RA3* also show coexpressed profiles during both ear and tassel development. In addition, genes that were differentially expressed in *ra1* and *ra3* mutants tended to be coexpressed with *RA1* and *RA3*. As a control for the mRNA-Seq data, we analyze dynamic changes in expression profiles of the Auxin response DR5::RFP.ER marker and additional developmental markers, such as pWUS1::NLS::RFP. Overlaying these marker profiles on the mRNA-Seq-based expression map will provide insight for linking dynamic changes in gene expression to hormonal signaling pathways in stem cells during meristem differentiation.

We are integrating the RNA-Seq data sets with other types of genomics data and further experimentation. We are currently analyzing DNA-occupancy maps of *RA1*, using chromatin immunoprecipitation followed

by high-throughput sequencing (ChIP-Seq). This will provide a genome-wide view of gene targets potentially regulated by *RA1*. Using these data, we can infer putative mechanisms of regulation acting through *RA1* to control branching in maize. We have also sequenced transcriptomes of comparable inflorescence stages in sorghum and will use these data in comparative analyses with the maize RNA-Seq data. These analyses will help in selecting candidate genes that act as key developmental regulators. The function of such candidate genes will be tested in follow-up genetic and molecular experiments.

Identification of Genes Participating in the RAMOSA3 Network

A. Goldshmidt, M. Pautler, D. Jackson

The characterization of molecular mechanisms controlling the branching of cereal inflorescences and the identification of novel genes that participate in this process are crucial for the improvement of yield traits of commercial crops. In our current research, we have been using maize mutants *ramosa1* (*ra1*), *ramosa2* (*ra2*), and *ramosa3* (*ra3*), which have increased branching in the inflorescences, in order to characterize molecular networks regulated by these genes. Ramosa3 is of particular interest, because it encodes a predicted metabolic enzyme, a trehalose phosphate phosphatase, rather than a transcription factor or signaling molecule that are more typical of developmental regulators. We have used a classic genetic approach—analysis of multiple mutant phenotypes—for identification of the relationships between *RAMOSA* genes and other genes involved in developmental networks. We first characterized double mutants of the *ramosa* genes with mutants involved in auxin transport (*barren inflorescence2* [*bif2*]) and in auxin synthesis (*sparse inflorescence1* [*spi1*]). Here, we found additive interactions, suggesting that networks regulated by the *RAMOSA* genes act in parallel to the auxin-related networks. Second, we characterized double mutants with mutants of genes involved in networks regulating meristem maintenance, such as *knotted1* (*kn1*), *fasciated ear2* (*fea2*), and *thick-tassel dwarf1* (*td1*). Here, our analysis revealed that phenotypes of the *ra1* and *ra2* mutants were enhanced by *kn1* loss-of-function mutations, whereas *ra3* mutant phenotypes were suppressed. *ra2* and *ra3* mutants showed additive interactions with *fea2*, whereas *fea2* phenotypes were suppressed by *ra1*, and all three *ramosa* mutants showed additive interactions with *td1*. These results indicate that *RAMOSA* gene activities are involved in networks regulating meristem maintenance. However, our results indicate that different *RAMOSA* genes may regulate determinacy by different mechanisms, some of which are overlapping and some distinct.

We also wish to gain a more detailed understanding of the *RAMOSA3* network using chemical mutagenesis followed by suppressor enhancer screens. So far, 850 segregating families were screened, and 46 suppressors and 17 enhancers were identified. Due to variability of the *ra3* mutant phenotype, it is important to verify enhancement/suppression in different environments. Therefore, all modifiers were regrown in at least two different fields for verification and crossed to *ra3* mutants in a different genetic background to generate mapping populations. Nine suppressor and three enhancer families have been verified and are being prepared for mapping. Interestingly, only one out of the nine suppressors and one out of the three enhancers showed single-mutant phenotypes, resulting in dwarfism and validating the use of the modifier approach. In the next year, we will focus on mapping and identification of these modifiers and characterize novel genes participating in the *RAMOSA3* network.

PUBLICATIONS

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PLANT DEVELOPMENTAL BIOLOGY, STEM CELLS, AND FLORAL BRANCHING SYSTEMS

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

Beauty and agricultural productivity in the Plant Kingdom is directly linked to the number and arrangement of branches and flowers on specialized shoots known as inflorescences. Inflorescence branching is determined by specialized groups of pluripotent stem cells at growing tips called shoot apical meristems (SAMs), which undergo reproductive "phase transitions" in response to environmental cues coinciding with innate molecular events. Inflorescences can be simple, producing only a single flower, or they can be highly complex, producing dozens of branches and hundreds of flowers. Our research is focused on the hypothesis that the rates at which SAMs undergo reproductive transitions dictate inflorescence diversity, which provides a basis for taxonomy as well as a primary point of selection in domestication and crop improvement. Little is known about the molecular dynamics regulating reproductive phase transitions, especially for species with different growth habits. In particular, one of the most fundamental, yet poorly understood, decisions is whether an inflorescence grows indefinitely (indeterminate growth) or if signals are received to end growth (determinate growth). Determinate plants produce the most elaborate inflorescence architectures, particularly among species having the widely deployed "sympodial" growth habit, represented by tomato. We are uniting genetics with quantitative genomics to discover the genes and networks that control sympodial reproductive phase transitions, which are a hallmark of perennial species, such as trees, and represent the growth habit of half of the world's flowering plants. We are taking advantage of several tools in tomato (*Solanum lycopersicum*) to understand how sympodial reiteratation is modulated to give rise to its multiflowered inflorescence, and what molecular mechanisms are responsible for variation in inflorescence branching in the larger nightshade (Solanaceae) family. Importantly, the Solanaceae comprises several crop and ornamental species, including potato, pepper, eggplant, petunia, and tobacco, as well as many edible wild species. Therefore, our research addresses questions that are relevant to evolution as well as agriculture.

In a related project, we are exploring the phenomenon of hybrid vigor, or heterosis, which occurs when different inbred plants are crossed to produce superior hybrids yielding more flowers, fruits, and seeds. Heterosis, first described by Darwin, is widespread in nature and is responsible for the dramatic and necessary increase in crop yields achieved over the last century. We are testing the hypothesis that heterosis can be caused by subtle changes in gene dosage originating from heterozygous single-gene mutations affecting growth and development. In a screen involving dozens of tomato heterozygous mutants, we have identified three heterosis genes, including a reproductive transition gene and an inflorescence branching gene. A conclusion from our findings during the last year is that there are several unknown genes that regulate reproductive transitions and inflorescence architecture, which are major factors influencing reproductive capacity and yield. Therefore, our continued efforts to expose new genes and networks affecting flowering in tomato will provide new targets for functional studies that can be applied to crop improvement.

Sympodial Growth in Tomato

Tomato is a powerful model to study mechanisms of sympodial reproductive transitions because it is composed of three distinct shoot systems, each of which undergoes a termination event (Fig. 1). The first termination originates from the embryonic meristem, which produces about eight leaves before ending growth with a multiflowered inflorescence. The next termination is in the sympodial shoot (SYM), which grows out from the last axillary meristem produced from the primary shoot. All SYMs develop three leaves before producing a final inflorescence, and each tomato shoot after the primary transition is derived from the indefinite reiteration of SYMs. The third termination occurs in the inflorescence, which produces about seven flowers in a zigzag arrangement. The inflorescence is also compound, consisting of reiterating sympodial inflorescence
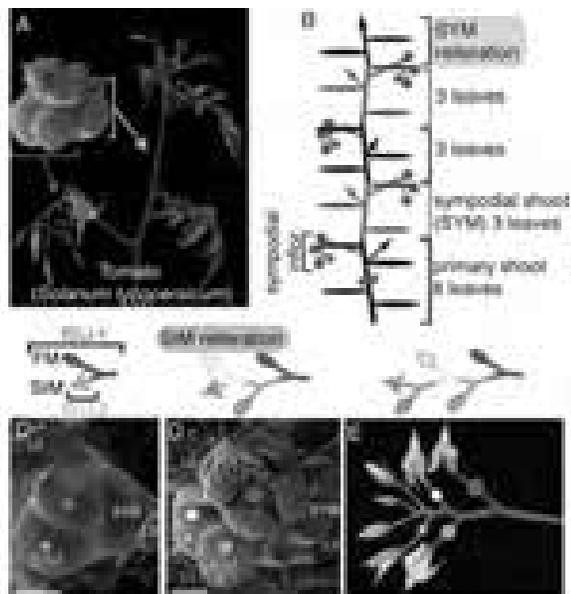
Figure 1. Determinate sympodial growth and floral reiteration in tomato. (*A*) A typical tomato plant and SEM image of meristems corresponding to the inflorescence and the vegetative shoot. (*B*) Schematic of modular sympodial shoot (SYM) growth and reiteration. (*C–E*) Sequential SEM images (*C–D*) and mature inflorescence (*E*) highlighting sympodial inflorescence meristem (SIM) reiteration.

meristems (SIMs). Finally, each axillary meristem produces primary, sympodial and axillary shoots and inflorescences. Thus, tomato growth is modular, and numerous reproductive transitions throughout life give rise to a plant with hundreds of branches, inflorescences, and flowers.

Two Major Genes Controlling Inflorescence Meristem Determinacy and Branching in Tomato

In previous work, we identified two inflorescence branching genes called *COMPOUND INFLORESCENCE* (*S*) and *ANANTHA* (*AN*). *S* produces normal fertile flowers as well as branching patterns that resemble wild species, suggesting that branching complexity in the Solanaceae family might be based on *S*, or the pathway in which it resides. *S* encodes a homeodomain transcription factor related to *Arabidopsis WUSCHEL HOMEOBOX 9* (*WOX9*) that, interestingly, has no effect on branching in inflorescences that grow continuously (indeterminate "monopodial" shoots). In contrast,

our genetic and molecular analyses have revealed that the transient sequential expression of *S* followed by *AN* promotes branch termination and flower formation in sympodial plants. Thus, these two genes are major regulators of the transition and reiteration of SIMs, such that when either gene is mutated, there is a delay in floral termination that allows for additional branches to develop. *S* is the key gene responsible for branching variation in domesticated tomatoes, as we have found that all highly branched varieties carry mutations in *S*, with most based on a classical mutant allele first described 150 years ago. Furthermore, our recent quantitative trait locus (QTL) genetic analyses on weak branching varieties of tomato show that at least one responsible gene resides on chromosome 2 in close proximity to *S*. We are now expanding genetic and molecular studies to explore whether *S* is responsible, at least in part, for the single inflorescence branching events (bifurcations) that are characteristic of several wild tomato species, such as *Styrax peruvianum*.

A Comparative Morphological Study of Solanaceae Inflorescence Development

K. Liberatore, S.-J. Park

How inflorescences develop and branch is only beginning to be studied in diverse species, and there is little known about how meristems elaborate in sympodial plants. To establish a morphological foundation for how sympodial inflorescence complexity arises, we are carrying out a comprehensive, comparative morphological study involving carefully selected Solanaceae representing a developmental continuum of inflorescence complexity. By combining scanning electron microscopy (SEM), in situ hybridization, and transgenic candidate gene knockdown experiments, we are deciphering the "rules" of SIM branching. In particular, we have compared *s* with wild tomato species and other Solanaceae to establish a developmental baseline on which to draw conclusions about how SIMs are initiated, reiterated, and branch. By screening more than 120 Solanaceae, we have selected a subset of species that represent a range of architecture types, including *Nicotiana benthamiana, Capsicum annuum* (pepper), and *Petunia nyctaginiflora* (petunia) (single-flower inflorescences), *Styrax cleistogamum* and *Sicotiana prinophyllum* (two flowers per inflorescence), *S. conglobatum* and *S. americanum* (tomato-like inflorescence), and *S. peruvianum* and *Nicotiana tobaccum* (branched, many flowers per inflorescence). We have already studied in detail the ontogeny of *N. benthamiana*

and *S. peruvianum*, which has revealed that primary transitions are faster and slower, respectively, compared to tomato. We have also developed an interest in assessing if there is any relationship between the level of branching and species-specific phyllotactic patterning. To address these questions, we are developing a series of marker genes fused to fluorescence proteins to generate reporter lines for studying sympodial transitions in four dimensions (time and space) using confocal and two-photon microscopy.

A Comparative Molecular Study of Inflorescence Development

K. Liberatore [in collaboration with X. Wu, University of Southern California]

As mentioned above, it is unclear to what extent reproductive transitions are similar or different in plants such as the monopodial *Arabidopsis* and the sympodial tomato. We are determining if branching genes such as *S* in tomato are functionally conserved across diverse species by using a series of transgenic knockdown tools (e.g., artificial microRNAs) to determine if *WOX9* has an unforeseen role in inflorescence development in *Arabidopsis*. Curiously, mutations in *Arabidopsis WOX9* have been characterized, but phenotypic defects seem to be restricted to the early stages of embryonic patterning. This early embryonic defect and resulting seedling lethality has prevented a more detailed analysis of later stages of development. By selectively targeting the elimination of *WOX9* transcripts, we hope to determine if and when *WOX9* functions to regulate inflorescence meristem elaboration. Our first set of experiments suggest targeting *WOX9* using RNA interference (RNAi) constructs driven by a floral-specific promoter causes the *Arabidopsis* floral meristem to branch in a way that is reminiscent of *s* mutations in tomato.

The Molecular Dynamics Underlying Sympodial Meristem Transitions in Tomato and Related Solanaceae

S.-J. Park, K. Jiang [in collaboration with M. Schatz, Cold Spring Harbor Laboratory]

We are taking advantage of the three types of meristems of tomato (primary shoot, SIM, and SYM) to elucidate the dynamic changes in gene expression that take place during reproductive transitions in plants, with a focus on determinate sympodial growth. Tomato meristems, like

other Solanaceae, are extremely large and easily dissected and collected in sufficient amounts for RNA extraction. Using a simple acetone fixation protocol we have developed this past year, we are growing uniform batches of wild-type plants and precisely harvesting meristems having no more than two leaf primordia every 3 days until floral termination. After the transition, we collect SYM, SIM, and FM (floral meristem) tissue. To date, we have completed gene expression analyses on seven stages of sympodial growth using quantitative deep mRNA sequencing (Illumina mRNA-Seq), and we have achieved excellent temporal resolution to reveal that reproductive transitions are highly quantitative and dynamic (Fig. 2). Furthermore, each meristem stage has its own molecular identity and age. We have been able to quantify meristem state using several algorithms, which predict that the SYM shares identity with the late vegetative meristem (LVM) stage from the primary shoot. This suggests that upon inception, SYMs are preprogrammed as if young leaves have already been initiated. These data provide an early clue to explain how sympodial shoots reiterate if they somehow sense the termination rate of the primary shoot, perhaps through changes in apical dominance, which then causes LVM genes to turn on early and strong in each initiating SYM. Interestingly, SIMs have dual identities, overlapping with the transition meristem (TM) and FM. The SYM and SIM also express their
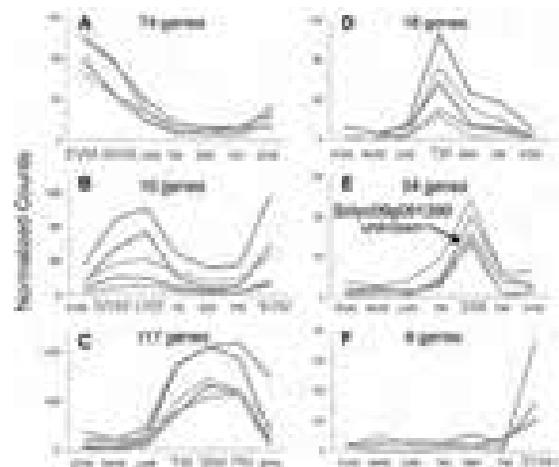
Figure 2. Six representative clusters of tomato gene expression dynamics showing gradual (*A–C*) and transient, stage-specific peaks (*D–F*) as sympodial meristems undergo termination. Data were grouped into 25 clusters (see text for details) and up to five representative genes are plotted out of the total in each cluster (indicated at top) (*A*). Early vegetative meristem (EVM) genes, (*B*) late vegetative meristem (LVM) genes, (*C*) reproductive (TM, SIM, FM) genes, (*D*) TM genes, (*E*) SIM genes, (*F*) SYM genes.

own stage-enriched genes, indicating that each meristem has unique identity. We are now performing quantitative transcriptomics on several Solanaceae species from a morphological allelic series to decipher the differential dynamics that determine whether a SIM exists, to what extent it reiterates, and whether it branches. To study differences in sympodial programs, our project requires that quantitative transcriptomics be performed on nonmodel *Solanaceae* to compare sympodial reproductive transitions. We have verified that other species' meristems can be harvested at similar temporal resolutions and, most important, that reference transcriptomes can be established to enable robust species-specific quantification by using *N. benthamiana*, a single-flowered species representing the most distantly related *Solanaceae* within our chosen species set. Our findings have thus far indicated that de novo transcriptome assembly and quantification in nonmodel *Solanaceae* are achievable and will prove powerful for comparing expression dynamics among diverse sympodial programs. These data are also providing a first list of candidate genes for functional studies using reverse genetic tools.

Novel Inflorescence Branching Mutants

C. MacAlister

To increase our knowledge of the genes and networks regulating sympodial inflorescence patterning, we are investigating new mutants with inflorescence architecture defects. In particular, we are characterizing and cloning the gene responsible for a new tomato mutant called *fasciated inflorescence* (*fin*), which produces a highly branched

Figure 3. New tomato mutant (fasciated inflorescence) produces greatly enlarged flowers and fruits.

inflorescence with greatly enlarged flowers and fruits with increased organs (Fig. 3). The phenotypes of *fin* mutants suggest some genes have dual roles in regulating determinacy, affecting both the inflorescence and floral meristems. In contrast, *s* and *an* seem to be specific to the inflorescence meristem. Indeed, our analysis of *fin:s* double mutants indicates that these genes operate in separate genetic pathways. We have cloned the *FIN* gene by uniting map-based cloning with mRNA-Seq and found that *FIN* encodes a protein of unknown function, lacking any known protein motifs. Mutants of *fin* are reminiscent of *clavata* mutants in *Arabidopsis*, except that *fin* produces an enlarged meristem early in the vegetative phase of development and continues to enlarge as the primary reproductive transition ensues. This results in an oblong enlarged meristem that produces leaves ectopically, alters phyllotaxy, and initiates several additional sympodial shoots (SYMs). In addition to standard molecular analysis, we are now studying an allelic series of *fin* mutants in more detail by (1) performing mRNA-Seq on *fin* meristems before and after the reproductive transition, (2) studying double-mutant combinations with other tomato inflorescence and flowering mutants, and (3) exploring phenotypic consequences of mutations in the orthologous genes of *Arabidopsis,* which is facilitated by *fin* being a member of a small gene family all the way down to moss.

We are also studying a classical mutation called *terminating flower* (*tmf*), which uniquely causes an early termination of the primary inflorescence meristem to generate a single terminal flower that fails to produce SIMs and SYMs. Interestingly, all axillary meristems develop normally, generating typical SIMs and SYMs. By taking advantage of our recent efforts to sequence the most closely related wild species of tomato, *S. pimpinellifolium*, we have map-based cloned the defective gene in *tmf* and found that it encodes yet another protein of unknown function. Our finding of several new genes controlling tomato inflorescence architecture highlights that novel mechanisms regulate determinate sympodial growth.

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

K. Jiang [in collaboration with D. Zamir, Hebrew University, Israel]

The genetic and molecular bases for how hybrid progeny outperform their parents (heterosis) have not been resolved. On the basis of classical studies in noncrop

plants and recent findings in yeast, we have tested the hypothesis that single-gene mutations cause phenotypic overdominance in the heterozygous state through a reduction in gene dosage. By taking advantage of a large "mutant library" in an isogenic cultivated tomato background (*Solanum lycopersicum*, cv. M82), we have identified multiple genes that show heterosis through overdominance. We have discovered that *SINGLE FLOWER TRUSS* (*SFT*), which is the genetic originator of the flowering hormone florigen that drives the reproductive transition in all plants, increases yield up to 60% when loss-of-function alleles are heterozygous. Yield overdominance from *sft* heterozygosity translates to several distinct genetic backgrounds and environments, and we show that several traits integrate pleiotropically to drive heterosis in a multiplicative manner. Our results suggest that a simple reduction in the dosage of a single gene can cause dramatic transgressive variation. These findings provide the first example of a single overdominant gene for yield, and they suggest that screening for mutations with heterozygous effects on growth can improve productivity in other agricultural organisms. On the basis of these findings, we have extended our studies to screen for other mutants that show heterosis in the heterozygous state. In collaboration with Dani Zamir in Israel, we have found that heterozygosity for the *compound inflorescence* mutant increases yield by causing 20%–40% of inflorescences to branch once (bifurcate),

without any pleiotropic effects on growth. Importantly, we have found that heterosis from *s/+* heterozygotes is heritable through meiosis and acts additively with other heterotic mutants, thereby opening the door for pyramiding heterosis genes to increase flower and fruit production even further. A fundamental question on single-gene heterosis is how subtle changes in gene dosage affect the transcriptional networks that drive phenotypic heterosis. Because of the greatly reduced genomic complexity of single-gene heterosis, we have begun performing targeted and global quantitative gene expression analyses to link heterotic effects with transcriptional changes in developmental pathways and larger transcriptional networks. To our surprise, we have found that the florigen-encoding *SFT*, which is believed to be expressed only in leaves [4, 5], is transcribed specifically in the TM and SIM meristem stages and overlaps with transcription of its antagonist *SP*. One intriguing possibility we will explore is whether local transcription and a changing balance of *SFT* and *SP* in transitioning meristems from *sft/+* heterozygotes is the basis for this form of heterosis.

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Ke Jiang

EPIGENETIC INHERITANCE AND REPROGRAMMING IN PLANTS AND FISSION YEAST

R. Martienssen   B. Arcangioli   K. Creasey   D. Irvine   J. Ren   M. Tanurdžić
             F. Borges   E. Ernst   Y. Jacob   A. Schorn   S. Thomain
             J. Calarco   P. Finigan   C. LeBlanc   R. Shen   F. van Ex
             S. Castel   J.-J. Han   U. Ramu   J. Simorowski   M. Vaughn
             A.-Y. Chang   K. Hansen   M. Regulski   C. Tang   M. Zaratiegui

Plants and fission yeast provide excellent model organisms to address the question of how epigenetic information is propagated to daughter cells, including transposon regulation, heterochromatic silencing, and gene imprinting, important both for plant breeding and for human health. We are investigating the inheritance of heterochromatic silencing and the role of RNA interference (RNAi) in the fission yeast *Schizosaccharomyces pombe* and the plant *Arabidopsis thaliana,* as well as continuing our work on *Zea mays.* In the past year, we have found that RNAi is important in determining germ cell fate as well as in transposon activation, whereas in fission yeast, transposable elements (TE) control DNA replication via the highly conserved centromere-binding protein B (CENP-B), which contributes to their epigenetic regulation.

This year, we welcomed postdocs Yannick Jacob and Jie Ren, computational biologist Evan Ernst, graduate student Stephane Castel, and research technician Chantal LeBlanc. We said farewell to Rulan Shen, who retired after more than 15 years in the plant group. Danielle Irvine accepted a faculty position at the Murdoch Children's Research Institute in Melbourne, Australia. Matt Vaughn left for a position at the Texas Advanced Computing Center at the University of Texas in Austin, and Klavs Hansen returned to Copenhagen. Filipe Borges was a visiting student from the Gulbenkian institute (Lisbon, Portugal), Sophie Thomain visited from École Normale Supèrieure, and Benoit Arcangioli joined the laboratory for 6 months on sabbatical from Institut Pasteur (Paris).

CENP-B Preserves Genome Integrity at Replication Forks Paused by the Retrotransposon LTR

M. Zaratiegui, M. Vaughn, D. Irvine [in collaboration with J. Bahler, University College London; B. Arcangioli, Institut Pasteur]

CENP-B is a widely conserved DNA-binding factor associated with heterochromatin and centromeric satellite repeats. In fission yeast, CENP-B homologs have been shown to silence long terminal repeat (LTR) retrotransposons by recruiting histone deacetylases. However, CENP-B factors also have unexplained roles in DNA replication. We have shown that a molecular function of CENP-B is to promote replication fork progression through the LTR. Mutants have increased genomic instability caused by replication fork blockage that depends on the DNA-binding factor switch-activating protein 1 (Sap1), which is directly recruited by the LTR. The loss of Sap1-dependent barrier activity allows the unhindered progression of the replication fork, but this results in rearrangements deleterious to the retrotransposon. We conclude that retrotransposons influence replication polarity through recruitment of Sap1 and transposition near replication fork blocks, whereas CENP-B counteracts this activity and promotes fork stability. Our results may account for the role of LTR in fragile sites and for the association of CENP-B with pericentromeric heterochromatin and tandem satellite repeats in mammalian genomes including humans.

RNAi Promotes Heterochromatic Histone Modification through Replication-Coupled Transcriptional Silencing

M. Zaratiegui, J. Ren, A.-Y. Chang, D. Irvine, S. Castel [in collaboration with F. Antequera, University of Salamanca, Spain; W.Z. Cande, University of California, Berkeley; B. Arcangioli, Institut Pasteur]

Heterochromatin comprises tightly compacted repetitive regions of eukaryotic chromosomes. It is inherited through mitosis and has roles in transcriptional silencing and genome integrity. In fission yeast, the epigenetic inheritance of heterochromatin requires RNAi to guide histone modification, which occurs during the DNA replication phase of the cell cycle. The alternating arrangement of origins of replication and small RNA clusters in fission yeast centromeres provokes the collision of the transcrip-
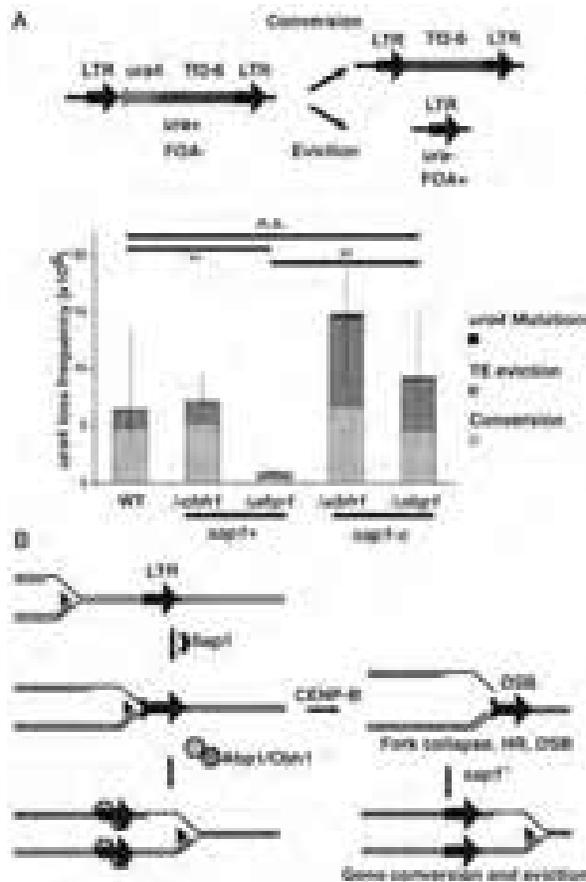
Figure 1. CENP-B and switch-activating protein 1 have opposite effects on retrotransposon Tf2 stability. (*A*) Ectopic recombination fluctuation assay. Strains with a reporter *ura4+* gene inserted in the Tf2-6 retrotransposon were plated onto 5-fluoro-orotic acid media that selects for *ura4* loss. Two potential mechanisms of *ura4* loss from the marked *Tf2-6::ura4* are indicated: gene conversion and eviction by LTR recombination. Columns represent total *ura4* loss frequency in wild-type, Δ*abp1*, Δ*cbh1*, Δ*abp1sap1-c,* and Δ*cbh1sap1-c* mutants, and shading indicates distribution of mode of ectopic recombination events in the *ura4* colonies obtained from wild-type (*n* = 93), Δ*abp1* (*n* = 88), Δ*cbh1* (*n* = 94), Δ*abp1sap1-c* (*n* = 91), and Δ*cbh1sap1-c* (*n* = 89) mutants. (*B*) Model for the interactions among Abp1, Cbh1, Sap1, and the replication fork at the LTR. In the triple mutant, replication of LTR retrotransposons in both directions results in gene conversion and eviction.

tion and replication machineries. We propose that RNAi promotes release of RNA polymerase (pol II), allowing completion of centromeric DNA replication by the leading-strand DNA polymerase, which recruits histone-modifying enzymes and spreads heterochromatin. In the absence of RNAi, stalled forks are repaired by homologous recombination without histone modification. Our

results may explain the participation of noncoding transcription and DNA replication in many systems of heterochromatin inheritance.

Comparative and Functional Analysis of Fission Yeast Genomes Reveals Conserved Regulation of Transposons by RNAi and CENP-B

K. Hansen, M. Zaratiegui [in collaboration with N. Rhind, University of Massachusetts]

Genome and small RNA sequencing from relatives of the fission yeast *S. pombe* has revealed a near extinction of transposons and the innovation of transposon-free centromeres in *S. pombe.* Small RNA corresponding to Gypsy class LTR retrotransposons were found in *S. japonicus,* but not in *S. pombe,* which correlates with the presence of LTR transposons in pericentromeric regions, where they presumably fulfill the same function as pericentromeric repeats in *S. pombe.* The *S. japonicus* genome lacks CENP-B proteins, suggesting that RNAi may have supplanted some of its functions, including transposon control and replication fork restart. Recent studies have found Dicer to be associated with LTR in *S. pombe,* even in the absence of abundant small interfering (siRNA), indicating a conserved function for transposon RNAi.

Role of RNAi in Epigenetic Inheritance and Plant Evolution

F. van Ex, K. Creasey, C. Tang [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory; R. deSalle, American Museum of Natural History; G. Coruzzi, New York University; D. Stevenson, New York Botanical Garden]

Using expressed sequence tag (EST) and genome sequence analysis, we identified proteins that have potential significance in the evolution of seed plants based on the direction of partitioned branch and hidden support on a 16-species tree, constructed from 2557 concatenated orthologous genes. In addition to basic metabolic functions, such as photosynthesis or hormones, genes involved in posttranscriptional regulation by small RNAs were significantly overrepresented in key nodes of the phylogeny of seed plants. For example, *Argonaute1* and the *RNA-dependent RNA polymerase 6* were found to be overrepresented in the angiosperm clade. We are exploring the functional basis of differences in the mutant phenotypes in rice (a monocot) and *Arabidopsis* (a dicot), as well as the potentially epigenetic consequences for floral development and germ cell fate.

Arabidopsis thaliana Chromosome 4 Replicates in Two Phases That Correlate with Chromatin State

M. Tanurdžić, M. Vaughn [in collaboration with L. Hanley-Bowdoin, B. Thompson, North Carolina State University]

We used flow cytometry and tiling microarrays to profile DNA replication of *Arabidopsis* chromosome 4 (chr4) during early, mid, and late S phase. Replication profiles for early and mid S phase were similar and encompassed the majority of the euchromatin. Late S phase exhibited a distinctly different profile that includes the remaining euchromatin and essentially all of the heterochromatin. Termination zones were consistent between experiments, allowing us to define 163 putative replicons on chromosome 4 that clustered into larger domains of predominately early or late replication. Early-replicating sequences, especially the initiation zones of early replicons, displayed a pattern of epigenetic modifications specifying an open chromatin conformation. Late replicons, and the termination zones of early replicons, showed an opposite pattern. Histone H3 acetylated on lysine 56 (H3K56ac) was enriched in early replicons, as well as the initiation zones of both early and late replicons. Replicon organization in *Arabidopsis* is strongly influenced by epigenetic modifications to histones and DNA.

Control of Female Gamete Formation by a Small RNA Pathway in *Arabidopsis*

F. van Ex, K. Creasey [in collaboration with J.-P. Vielle-Calzada, Langebio, CINVESTAV, Mexico]

In the ovules of most sexual flowering plants, female gametogenesis is initiated from a single surviving gametic cell, the functional megaspore, formed after meiosis of the somatically derived megaspore mother cell (MMC). The *Arabidopsis* protein ARGONAUTE 9 (AGO9) controls female gamete formation by restricting the specification of gametophyte precursors in a dosage-dependent, non-cell-autonomous manner. Mutations in *AGO9* lead to the differentiation of multiple gametic cells that are able to initiate gametogenesis. Mutations in *RNA-DEPENDENT RNA POLYMERASE 6* exhibit a defect identical to that of *ago9* mutants, indicating that the movement of small RNA silencing out of somatic companion cells is necessary for controlling the specification of gametic cells. AGO9 preferentially interacts with 24-nucleotide small RNAs derived from TEs, and

its activity is necessary to silence TEs in female gametes and their accessory cells. Our results show that AGO9-dependent small RNA silencing is crucial to specify cell fate in the *Arabidopsis* ovule and that epigenetic reprogramming in companion cells is necessary for small-RNA-dependent silencing in plant gametes.

Small RNA and Epigenetic Reprogramming in the *Arabidopsis* Male Germline

Y. Jacob, J. Calarco, C. Tang, C. LeBlanc, F. Borges [in collaboration with J. Becker, J. Feijó, Gulbenkian Institute, Lisbon, Portugal]

Most of the core proteins involved in the microRNA (miRNA) pathway in plants have been identified, and hundreds of miRNA sequences processed in the *Arabidopsis* sporophyte have been discovered by exploiting next-generation sequencing technologies. On the basis of comparative analysis of miRNAs identified in sperm cells by in-depth sequencing, 25 potentially novel miRNAs processed in sperm cells and pollen were identified, as well as enriched variations in the sequence length of known miRNAs, which might indicate subfunctionalization by association with a putative germline-specific Argonaute complex. ARGONAUTE 5 (AGO5), by close homology with AGO1 and localizing preferentially to the sperm cell cytoplasm in mature pollen, may be part of such a complex, as might 21-nucleotide "epigenetically activated" siRNAs that accumulate in sperm. We are examining epigenetic modifications of the genome during pollen development and how these might influence the profile of small RNA.

Transposon Instability in Interspecies Hybrids Is Mediated by RNAi

M. Tanurdžić, P. Finigan, E. Ernst, M. Vaughn [in collaboration with L. Comai, University of California, Davis; B.C. Meyers, University of Delaware; R.W. Doerge, Purdue University]

Silencing of transposable elements ensures genome stability and lowers the mutagenic effects of transposition. We have shown that COPIA retrotransposons from the pollen parent are activated in *Arabidopsis* interspecific hybrids, but only when matching 24-nucleotide siRNA are absent from the seed parent. Activated elements lose DNA methylation, and hybrid leaves accumulate 21-nucleotide "epigenetically activated siRNA" (easiRNA) normally found in pollen, along with RNA and extrachromosomal cDNA transposition intermediates. Our

results indicate that small RNA from the germline provides a general recognition mechanism for transposons in interspecific hybrids, reminiscent of hybrid dysgenesis and hybrid lethality in *Drosophila*, and may contribute to postfertilization hybridization barriers in a similar way.

Sequencing the Maize Methylome

M. Regulski [in collaboration with J. Kendall, D. McCombie, J. Hicks, D. Ware, Cold Spring Harbor Laboratory; S. Tingey, A. Rafalski, DuPont/Pioneer Genetic Discovery]

We have sequenced the methylome of B73 and Mo17 inbreds at 28.7- and 19.7-fold coverage, respectively, using bisulphite conversion and Illumina next-generation sequencing. In agreement with previous results, we have found that the vast majority of cytosine methylation in all three contexts (CG, CXG, and CHH) are found in intergenic regions and transposable elements, with exons containing only 5%–10% CG methylation. CHH methylation is rare and confined mostly to intergenic regions. Exemplars of transposable elements that differ in DNA methylation have been found, and variation between Mo17 and B73 was detected in regions of primary sequence similarity. Strategies for determining the inheritance and biological significance of epigenetic variation are being developed.

The Maize Mutant Tunicate1 Is Caused by Ectopic Expression of a MADS-Box Transcription Factor, Zmm19, in a Dosage-Dependent Manner

J.-J. Han [in collaboration with M. Aukerman, DuPont Crop Genetics; D. Jackson, Cold Spring Harbor Laboratory]

Pod corn was once regarded as the ancestral form of cultivated corn and was prized by pre-Columbian peoples for its magical properties. The Tunicate1 (Tu1) mutant of maize is a naturally occurring dominant mutation, resulting in a striking pod corn phenotype. The Zmm19 MADS box gene maps close to Tu1 and was previously sequenced from Tu1 mutants and half-tunicate derivatives (T. Meunster, G. Thiessen, unpubl.). This analysis revealed that Tu1 has duplicated copies of Zmm19 interrupted by a novel mutator-like DNA transposon in the 5′UTR (untranslated region), causing misexpression, but fine mapping proved impossible. We have found that yellow fluorescent protein (YFP)- and red fluorescent protein (RFP)-tagged

Zmm19 transgenic maize plants phenocopy Tu1. Moreover, misexpression of miR172 is detected in Tu1, suggesting that Zmm19 may target the miR172 gene, a conclusion supported by the phenotype of miR172 transgenic lines. The 22-Mb region surrounding the *tu1* locus in inbred B73 was selected for finishing during the maize genome sequencing project, and we have found that insertion of the mutator-like element resulted in a 1.8-Mb inversion immediately upstream of Zmm19 in Tu1. This rearrangement prohibits recombination, and so likely accompanied the origin of this ancient mutation.

Aquatic Plants as Biofuel Feedstocks

E. Ernst, M. Regulski [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory; J. Shanklin, Brookhaven National Laboratory; V. Citovsky, SUNY Stony Brook]

Aquatic flowering plants, such as common duckweed, have considerable potential as biofuel feedstocks due to their very rapid growth and high carbon accumulation per unit mass. Work is currently under way to assemble a draft of the 450-Mb *Lemna gibba* genome. 300 million Illumina GAIIx paired-end reads from short insert and mate pair libraries have contributed to an initial assembly of 300 Mb in contigs over 1 kb, with the largest reaching 250 kb. In addition, we obtained sequences from both small RNA and gene transcript libraries, which have already been used to identify 10 genes central to the oil biosynthesis pathway. These two libraries also enabled cloning native miRNA precursors to implement artificial miRNA gene silencing in *Lemna*. We successfully induced flowering in cultured common duckweed and continue to explore methods for large-scale seed production. Advances in transformation made this year by Brookhaven National Laboratory in conjunction with the progress in sequencing the genome and transcriptome should lead to the development of duckweed strains tailored to the production of lipid biofuels.

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Kate Creasey

PLANT DEVELOPMENTAL GENETICS

M. Timmermans   A. Benkovics   C. Fernandez-Marco   A. Husbands   K. Petsch
                     C. Clendaniel   A. Gal-on   M. Javelle   E. Plavskin
                     M. Dotto   C. Hu   M. Lodha   D. Skopelitis

Specification of adaxial–abaxial (upper/lower) polarity in the developing leaf drives the flattened outgrowth of the leaf blade and directs the differentiation of distinct cell types within the leaf's adaxial/top and abaxial/bottom domains. Both are important innovations in the evolution of land plants that maximize photosynthesis while minimizing water loss to the environment. In addition to being a key developmental process, adaxial–abaxial patterning is of particular interest from a mechanistic point of view and has proven to be an excellent model to study small RNA-mediated gene regulation. We previously showed that patterning of this developmental axis involves a cascade of opposing small RNAs, in which microRNA (miRNA) miR390 triggers the biogenesis of the *TAS3*-derived 21-nucleotide tasiR-ARF on the adaxial side of developing leaves. These confine the accumulation of abaxial determinants, including AUXIN RESPONSE FACTOR3 (ARF3) and miR166, to the lower side of leaves. miR166, in turn, demarcates the abaxial side by repressing expression of class III homeodomain leucine zipper (HD-ZIPIII) transcription factors that specify adaxial fate.

Importantly, our analysis of the *TAS3 trans*-acting short interfering RNA (tasiRNA) pathway in *Arabidopsis* demonstrated that tasiR-ARF functions as a mobile positional signal in adaxial–abaxial patterning. Movement of this small RNA from its defined source of biogenesis in the two 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.

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 to act as instruc-

tive signals in development and 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. Previously generated lines, expressing artificial miRNAs from the epidermis, against *PHYTOENE DESATURASE* (*PDS*) resulted in a range of photobleaching phenotypes, due to silencing of *PDS* in the subepidermal mesophyll layers, consistent with small RNA mobility from the epidermis into underlying cells. To further investigate miRNA mobility in multiple distinct developmental contexts, we are expressing miRNAs targeting cell-autonomous GUS and GFP reporters from a number of promoters that drive expression in various spatiotemporal patterns. A first analysis showed that miRNAs expressed from the vascular-specific *SUC2* and *ATHB8* promoters lead to miRNA-mediated gene silencing in the surrounding mesophyll and in the pavement cells of the epidermis, consistent with the idea that miRNAs can traffic from the vasculature into adjacent cells. Interestingly, no reporter silencing was observed in the trichomes of the epidermis, which are symplastically isolated. The tissue specificity of the promoters was verified using transgenic lines that express the respective transcriptional fusions to the GUS reporter gene, and the accumulation of the artificial miRNAs is being monitored by in situ hybridization. Genetic resources to similarly investigate properties of tasiRNA mobility are also being generated. In addition, with the knowledge that miRNAs and tasiRNAs can traffic from the epidermis into underlying tissue layers, we are carrying out forward genetic screens to identify factors influencing this process.

The START Domain Regulates HD-ZIPIII Activity and Organ Polarity
A. Husbands

On the basis of our previous observations regarding the expression and function of miR166 and tasiR-ARF, it is evident that adaxial–abaxial patterning involves a cascade of positional signals. The mobile signals that pattern the

newly formed leaf are distinct from those that maintain polarity during subsequent development. We have recently started a new project to identify additional signals in adaxial–abaxial patterning. Defining the identity of mobile positional signals remains not only a pressing question in the field of organ polarity, but also one of the main challenges in contemporary plant development.

The adaxial-promoting HD-ZIPIII transcription factors contain a predicted START lipid-binding domain (Fig. 1A). Modeling of the START domain of the HD-ZIPIII member PHABULOSA (PHB) suggests structural similarity to human PC-TP, a phosphatidylcholine-binding START domain (Fig. 1B,C). This model and sequence conservation with other START domains was used to predict amino acids in PHB critical for START domain ligand binding. To assess the role of the START domain to PHB function, we generated transgenic *Arabidopsis* lines that express START domain variants of a PHB–YFP fusion protein. These PHB-YFP reporters also carry silent mutations in the miR166-target site, which normally leads to the formation of severely adaxialized leaves (Fig. 1D–G). Expression of a PHB-YFP variant, in which residues lining the ligand-binding pocket in the START domain are mutated (Fig. 1C), yielded plants with less-severe leaf phenotypes, whereas lines expressing a PHB-YFP variant with a deletion of the START domain resemble wild-type plants. These findings reveal positive regulation of HD-ZIPIII activity by an unknown ligand.

Experiments to determine the mechanism via which the START domain controls HD-ZIPIII function are ongoing. Ligand binding does not appear to affect the subcellular localization of these transcription factors, as confocal imaging showed that the PHB-YFP derivatives correctly localize to the nucleus. We are currently investigating whether ligand binding affects PHB's ability to bind DNA, activate transcription, or interact with required protein partners. We have also developed a yeast-based assay that allows the high-throughput screening of chemical libraries for compounds that are bound by the HD-ZIPIII START domain and modulate protein activity. Compounds identified in this screen may not only provide critical insight into the nature of the HD-ZIPIII ligand, but also identify agonists/antagonists of the START domain that enable innovative experimentation into the mechanism via which HD-ZIPIII proteins regulate diverse aspects of plant development, including stem cell activity and organ polarity.

Characterization of Novel tasiRNA Loci in Maize

M. Dotto [in collaboration with M. Aukerman, M. Beatty, R. Meeley, DuPont-Pioneer]

tasiRNAs are processed from noncoding *TAS* precursor transcripts. Following cleavage by a miRNA-loaded
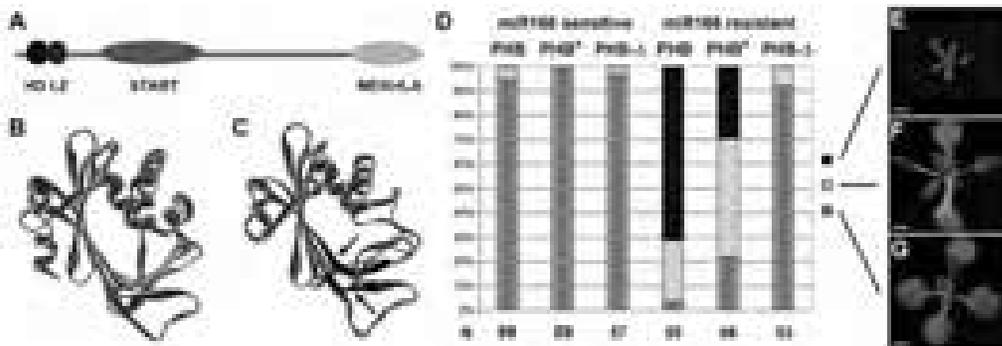
Figure 1. Activity of the HD-ZIPIII transcription factors is regulated by the START lipid-binding domain. (*A*) Schematic representation of the domain structure of HD-ZIPIII proteins. (HD) Homeodomain, (LZ) leucine zipper, (START) START lipid-binding domain, (MEHKLA) MEKHLA/PAS transcription regulatory domain. (*B*) Representation of the structure of the human PC-TP START domain modeled in SwissExpasy. (*C*) Predicted fold of the PHB START domain modeled in SwissExpasy. Amino acids mutated in the PHB-YFP derivative are highlighted in black (arrows). (*D*) Distribution of phenotypes observed in plants transformed with various PHB derivatives that either remain under control of miR166 (*left*) or carry mutations that render the transcripts resistant to miR166 (*right*). (PHB) Full-length PHB protein; (PHB*) PHB derivative with critical amino acids in the START ligand-binding pocket mutated; (PHB-Δ) PHB derivative with the START domain deleted; (N) number of transformants analyzed. (*E–G*) Phenotypes in the transformants were grouped into three categories: (*E*) severely adaxialized, indicated in black, (*F*) weakly adaxialized, indicated in light gray, (*G*) largely normal, indicated in dark gray. Mutation or deletion of the START domain suppresses the ability of miR166-resistant PHB to adaxialize developing leaves.

ARGONAUTE (AGO) complex, one of the cleavage products is converted into double-stranded RNA through the activities of RNA-DEPENDENT RNA POLYMERASE6 (RDR6) and LEAF BLADELESS1 (LBL1/SGS3) and subsequently processed by DICER-LIKE4 (DCL4) into phased 21-nucleotide siRNAs. *Arabidopsis* contains four characterized *TAS* gene families. AGO1 is required for the biogenesis of tasiRNAs from the *TAS1*, *TAS2*, and *TAS4* loci, whereas miR390-loaded AGO7 triggers the biogenesis of *TAS3* tasiRNAs. We previously identified four *TAS3* loci in the maize genome; however, the *TAS1*, *TAS2*, and *TAS4* loci are not conserved between *Arabidopsis* and maize. To identify potential novel *TAS* loci in maize, we used a deep-sequencing approach to compare the small RNA content between wild-type and *lbl1* mutant apices. This identified four novel *TAS* candidates, all of which are targeted by miR390 and generate tasiRNAs closely related to the known tasiR-ARFs. We are currently using bioinformatics approaches to identify additional phased small RNA clusters in an attempt to determine whether these *TAS3* loci are the only phased siRNA generating loci in the maize genome.

In a separate approach, we are characterizing small RNAs that associate with specific AGO proteins. The maize genome encodes 20 AGO proteins, including one AGO7 and four AGO1 homologs. We are generating transgenic maize plants expressing FLAG-tagged versions of ZmAGO7 and ZmAGO1b to isolate associated small RNAs via immunoprecipitation. We expect to identify novel tasiRNAs, miRNAs, and possibly the transcripts that are targets of these small RNAs. For additional information regarding the small RNA pathways in maize, we are also characterizing small RNAs that associate with ZmAGO10. This AGO protein has been implicated in the miRNA- and tasiRNA-mediated regulation of meristem maintenance and leaf development in *Arabidopsis*. Phylogenetic analysis showed that the maize genome contains two close homologs of AtAGO10. Transposon insertion alleles for both genes have been identified, and we are currently assessing possible contributions of the ZmAGO10 homologs to meristem function and leaf development in maize. We analyzed the small RNAs associated with ZmAGO10 by deep sequencing the small RNAs present in immunoprecipitates with a ZmAGO10-specific peptide antibody. We found that ZmAGO10 proteins preferentially bind 21-nucleotide small RNAs containing a 5′-U, which are features common to nearly all plant miRNAs. However, the immunoprecipitation fraction was enriched for just a subset of known miRNAs and tasiRNAs, and these include miR166 and tasiR-ARF,

which we had previously shown to function in the specification of maize leaf polarity. A previously uncharacterized miRNA was most enriched in the immunoprecipitation fraction. In situ hybridization showed that this miRNA also accumulates in a polar pattern, establishing a gradient on the adaxial side of developing leaves, presenting the possibility that organ polarity is also regulated by a fourth small RNA signal. Screens for mutations affecting this miRNA and its targets are ongoing.

Small RNA-Regulated Gene Networks in Adaxial–Abaxial Patterning

K. Petsch

Mutants that disrupt tasiRNA biogenesis exhibit defects in leaf polarity, which are characterized by sectors of abaxial identity on the adaxial leaf surface and/or by radialization of the leaves. In maize, the severity of these phenotypes is largely dependent on the inbred background, with specific inbreds, e.g., A619, Mo17, and W22, typically enhancing the defects of tasiRNA biogenesis mutants, whereas other backgrounds, e.g., B73, lead to a weaker phenotype. This inherent diversity across different maize inbreds provides a useful tool to discover novel enhancers/suppressors of small RNA biogenesis or function, as well as inbred-specific targets of the tasiRNA pathway. We are using the severe *lbl1-rgd1* mutant allele of *lbl1* to identify such genetic modifiers. The *lbl1-rgd1* is embryo-lethal in W22, but it develops into a viable seedling in B73. Genetic analysis indicates the presence of at least one major modifier locus on chromosome 8 that is dominant in the W22 background. Currently, we are using mapping populations to fine map this inbred-specific enhancer/suppressor. In addition, we are performing a transcriptomics analysis of *lbl1-rgd1* mutant embryos and nonmutant siblings in the W22 and B73 backgrounds. Similar experiments are also in progress to characterize genetic modifiers of *dcl4* mutants. By combining these approaches, we hope to achieve a better resolution of the factors contributing to natural variation in the tasiRNA pathway.

Adaxial–Abaxial Patterning by the AS1-AS2 Complex

A. Husbands, A. Benkovics

Our findings indicate that establishment of organ polarity requires the precise spatiotemporal accumulation and relative efficacy of tasiR-ARF and miR166. We are using genetic and biochemical approaches to identify genes

that regulate the accumulation and activities of these polarizing small RNAs. We have previously shown that the *Arabidopsis* DNA-binding proteins ASYMMETRIC LEAVES1 (AS1) and AS2 form a complex that contributes to organ polarity. Using chromatin immunoprecipitation (ChIP), we have identified several polarity determinants that are direct targets of the AS1-AS2 complex. These include selected *MIR166* precursors as well as components of the *TAS3* tasiRNA pathway. Our ChIP results have been verified using reporter constructs and reverse-transcriptase–polymerase chain reaction (RT-PCR) analysis, and we are currently using ChIP-Seq to examine AS1-AS2 targets in a genome-wide context.

The abaxial determinants *ARF3* and *ARF4* are targets of the *TAS3*-derived tasiR-ARFs. We have shown that transcript levels for both *ARF* genes are up-regulated in *as2*. Considering that tasiRNA pathway components are direct targets of AS1-AS2, these transcription factors may control *ARF3* and *ARF4* expression through regulation of tasiR-ARF accumulation. To test the interaction between the AS1/AS2 and tasiR-ARF pathways, we generated *as2* plants expressing a tasiR-ARF-insensitive allele of *ARF3* (*ARF3-D*). Interestingly, double mutants develop highly serrated leaves with ectopic leaf-like outgrowths at the proximal margins. Removal of *ARF3* activity results in complete suppression of leaflet formation, as does the chemical disruption of polar auxin transport. These and other data led us to propose that leaflet formation occurs at auxin maxima in response to ARF3 activity. Confirming this, *as2 ARF3-D* plants expressing the PIN1:GFP polar auxin transport reporter show colocalization of auxin maxima with sites of incipient leaflet formation and prolonged activity of this reporter. We are currently examining the putative connection among the tasiR-ARF pathway, auxin transport, and the production of compound leaf architecture.

The Ancestral Role of the tasiRNA Pathway

E. Plavskin [in collaboration with R. Quatrano, Washington University, St. Louis, Missouri; M. Hasebe, National Institute for Basic Biology, Okazaki, Japan]

The diversity of multicellular organisms raises the question of how so many varied morphologies evolved. A number of studies have demonstrated that novel structures often arise through the hijacking of existing developmental pathways for new functions. The pathways controlling leaf development seem to be no exception, as many of these have been shown to be conserved in the moss *Physcomitrella patens*, whose ancestors diverged from the lineage of flowering plants ~100 million years before leaves first evolved

in the latter. Thus, studying the role of leaf polarity pathways in moss provides a unique opportunity to explore the evolution of complex novel structures.

We are focusing on the miR390-dependent tasiRNA pathway, which regulates expression of the abaxial determinants *ARF3* and *ARF4*. The genes involved in biogenesis of tasiRNAs, including *SGS3*, *RDR6*, and *DCL4*, and the tasiRNA targets are conserved among maize, *Arabidopsis*, and *P. patens*. Elucidating the function of this pathway in *Physcomitrella* may lead to an understanding of its ancestral role in plant development. To dissect the developmental processes in *P. patens* regulated by tasiR-ARFs, we are taking advantage of the unique ability of this moss to be transformed by homologous recombination and characterizing knockouts of genes involved in tasiRNA biogenesis, as well as the targets of the moss tasiR-ARFs. Our preliminary results indicate a role for tasiRNA regulation in filamentous stem cell division in moss. *sgs3* loss-of-function mutants display increased branching in the filamentous stage of growth, and mutants of some tasiRNA targets are lethal, potentially reflecting a defect in the ability of filamentous stem cells to divide. These results are further supported by a transcriptional reporter of *miR390* in moss, which shows expression in dividing cells. Because the miR390-dependent tasiRNA pathway is also involved in meristem maintenance, our preliminary results suggest a conserved role for the pathway in plant stem cell function. We are continuing to explore the function of miR390-dependent tasiRNA biogenesis in moss through the generation of additional knockouts and expression reporters. We have also begun exploring the function of a miRNA regulatory site in tasiRNA targets, which presents the unique opportunity to study the coregulation of a gene by a tasiRNA and miRNA.

Establishment of Determinacy during Organ Development

M. Lohda, C. Clendaniel

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. Our project in the lab aims to dissect the genetic networks that regulate stem cell homeostasis and that distinguish indeter-

minate stem cells from their differentiating derivatives.

Stem cell activity in the SAM is maintained in part by the class I *KNOX* homeobox genes. To give rise to differentiating structures, such as leaves, *KNOX* gene expression needs to be maintained in a stable "off" state throughout lateral organ development. We have previously shown that this process is mediated by the transcription factors AS1 and AS2, which form heterodimers that bind specific sites in the *KNOX* promoters and recruit the chromatin-remodeling factor HIRA to these loci in developing leaves. We have now identified a role for a Polycomb repressive complex 2 (PRC2) in the stable silencing of *KNOX* genes during leaf development. PRC2 complexes have histone H3K27 trimethylation (H3K27me3) activity, and we have identified this repressive mark on nucleosomes at the *BP* and *KNAT2* loci in leaves of *Arabidopsis*. Mutations in the PRC2 component CURLY LEAF (CLF) lead to reduced levels of this repressive H3K27me3 chromatin mark at the *KNOX* loci and to ectopic expression of *KNOX* genes in developing leaves. Likewise, H3K27me3 levels at the *KNOX* loci are dramatically reduced in the *as1* and *as2* mutants, identifying these DNA-binding proteins as upstream components in the *KNOX* silencing pathway and possible recruitment factors for the PRC2 complex. We have used biomolecular fluorescence complementation (BiFC) assays to study possible direct interactions between AS1 or AS2 and the PRC2 subunits. This assay revealed that AS1 but not AS2 interacts with two of the PRC2 subunits. Similar assays also revealed a direct interaction between HIRA and PRC2 components. To complement these BiFC assays, we are performing immunoprecipitation assays using an epitope-tagged version of the PRC2 component FIE, which will test an interaction among PRC2, AS1, and the chromatin-remodeling factor HIRA in vivo. In addition, tagged FIE is being used in chromatin immunoprecipitation assays to show direct binding of PRC2 at *BP* and *KNAT2*. In the future, these same FIE-HA lines will be used to study genome-wide targets of PRC2 by ChIP-Seq.

We have also established a role for the polycomb repressive complex 1 (PRC1) in the stable silencing of *KNOX* genes during leaf development. PRC1 is a downstream component in the polycomb pathway that maintains long-term silencing. In plants, this complex contains LIKE HETEROCHROMATIN PROTEIN1 (LHP1), which recognizes the H3K27me3 signature deposited by PRC2. We found that LHP1 directly binds silenced *KNOX* loci, and as expected, this binding is partially lost in an *as2* mutant. These data demonstrate that cellular differentiation is achieved via an epigenetic mechanism in which the AS1-AS2 complex serves to recruit Polycomb group proteins to pluripotency factors to suppress their expression during organ differentiation.

Small RNA-Regulated Gene Networks in SAM Function

M. Javelle

A number of key regulatory genes involved in meristem indeterminacy and organ initiation are under the control of miRNAs. To gain insight into small RNA-controlled gene networks required for SAM function and organogenesis, we are characterizing the precise expression patterns of selected mature miRNAs by in situ hybridization. Six miRNAs (*miR394*, *miR528*, *miR160*, *miR167*, *miR164*, and *miR319*) have been analyzed thus far. Each miRNA shows a distinct expression pattern, suggesting diverse contributions of small RNAs and the pathways they target to the regulation of SAM function. The observed expression patterns suggest that nearly all six miRNAs have functions associated with leaf initiation and leaf development. Two of these small RNAs show a polar pattern of expression in developing leaves, presenting the possibility that maintenance of organ polarity is regulated by additional mobile small RNA signals. In addition, we used laser microdissection to collect cells from distinct functional domains within the SAM and developing leaf primordia of maize to analyze the expression profiles of the small RNA precursors and downstream targets. Cells from 12 distinct regions of the shoot apex—including the epidermal and subepidermal layers of the SAM, the stem-cell-containing tip of the SAM and determinate leaf primordia P0 to P3, the abaxial and adaxial sides of young leaf primordia, and tissues in the vasculature and stem—were collected separately. The quality of RNA isolated from these tissue samples has been verified using known marker genes. A first expression analysis of miRNA precursor genes revealed that complex regulatory mechanisms underlie the generation of the distinct miRNA expression patterns. These RNA samples will also be analyzed by deep sequencing to compare the global gene expression profiles of these distinct cell populations in the SAM and developing leaf primordia.

Characterization of *chups*, an Embryonic Patterning Mutant in *Arabidopsis*

C. Fernandez-Marco

Embryogenesis in *Arabidopsis* proceeds through a series of highly stereotyped divisions and gives rise to a struc-

ture that contains two stem cell niches—the shoot apical meristem and the root apical meristem—which are the sources of all cells for postembryonic development. An asymmetric division of the zygote gives rise to the apical embryonic lineage and the basal extra-embryonic suspensor. After several more rounds of cell division, the upper tier of the embryo generates the shoot apical meristem, whereas the upper cell of the suspensor undergoes two successive asymmetric divisions to give rise to the root apical meristem. We have identified a mutant that delays embryonic development. At the time that wild-type embryos have reached maturity, mutant embryos show globular or heart stage morphologies. We have called this mutant *chupa chups* (*chups*), which means lollipop in Spanish, as a reminder of the globular shape of mutant embryos. Despite the delay in embryogenesis, patterning of the shoot apical meristem is unaffected in *chups* mutants. In contrast, cell division of the hypophysis, the specialized cell of the suspensor that ultimately gives rise to the quiescent center of the root stem cell niche, is perturbed in *chups*. Auxin transport and signaling are key to the specification of the hypophysis and patterning of the root stem cell niche. Preliminary data show that expression of markers for auxin transport and signaling are altered in *chups* and that an

auxin maxima is no longer established at the boundary between the proembryo and suspensor, which affects specification of the hypophysal cell. Although *chups* mutant embryos are not normally viable, mutants can be rescued by transferring fertilized ovules onto culture medium. Consistent with the above-mentioned defects, rescued mutant seedlings lack a root. *chups* maps to the top arm of chromosome IV. We are currently determining the underlying mutation. The outcome of these experiments will allow us to characterize and position CHUPA CHUPS in the genetic network that patterns the root stem cell niche during embryogenesis.

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Eugene Plavskin

GENOMICS

The genomics program is composed of faculty working across disciplines and research areas. Their main research interests are genomic organization, structural variation of the human genome as related to disease, computational genomics and transcriptional modeling, and sequencing technology. The genomics faculty is located at both the main campus and the Woodbury Genome Center, which is located a few miles from the main campus. Faculty in the genomics program conduct research in the areas of human genetics, functional genomics, small RNA biology, and bioinformatics.

Thomas Gingeras and colleagues study where and how functional information is stored in genomes. These efforts help us to 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 modENCODE (model genome ENCODE) projects of the National Institutes of Health. His research has revealed that almost the entire lengths of genomes in organisms ranging from bacteria to humans can be transcribed into RNA and that most RNA products are not destined to be translated into proteins. In fact, noncoding RNAs (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. Others serve as scaffolds upon which large protein complexes are assembled. This year, Gingeras and colleagues published 18 papers detailing results of the modENCODE project to date. With these, the annotated portions of the *Drosophila* and *Caenorhabditis elegans* genomes have now grown severalfold, bringing functional regulatory networks into view in unprecedented detail.

The insights of W. Richard McCombie and colleagues have led to the introduction and optimization of novel methods of high-throughput genome sequencing. His team has made it possible to catalog variation among individual organisms in a way that would have been unthinkable a decade ago. They have brought online a new generation of Solexa sequencers and optimized their function to a level at which 10 billion DNA bases can be sequenced in a typical day, and on some days, as many as 20 billion. McCombie's team has been involved in international efforts culminating in genome sequences for maize and rice, two of the world's most important food crops. They have also had an important role in projects to sequence the flowering plant *Arabidopsis thaliana*, the fission yeast *Schizosaccharomyces pombe*, and *Homo sapiens*. In 2010, they contributed to the sequencing of *Theobroma cacao*, the tree variety whose seeds are used to make the world's finest chocolate. McCombie's group is currently involved in other important projects to sequence genes of special interest to human health, including *DISC1*, a strong candidate gene for schizophrenia, as well as genomic regions likely implicated in bipolar illness. In collaboration with the Memorial Sloan-Kettering Cancer Center, the group is using a method called hybrid resequencing, developed with Greg Hannon, to look at mutations in samples collected from patients with prostate cancer.

Lincoln Stein's lab is developing databases, data analysis tools, and user interfaces to organize, manage, and visualize the vast body of information generated by biologists. They run the modENCODE (model genome ENCODE) Data Coordination Center, which is responsible for collecting, integrating, and publishing the information collected by the ENCODE (ENCyclopedia of DNA Elements) consortium in a form that can be extended and combined with information from other human and model organism genome databases, and they contributed to two major modENCODE-papers published in *Science* in December 2010. The group also develops algorithms for identifying key actors in cancer genomes as well as software for identifying aberrant splicing and expression of genes in cancers. Stein's group manages and curates the WormBase and Reactome databases and is involved in the iPlant Collaborative, an effort to better enable plant biology researchers to collaborate in cyberspace.

Using multidisciplinary approaches that combine computational analysis, modeling, and prediction with experimental verification, Doreen Ware's lab seeks a deeper understanding of the evo-

lution 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? Ware's team also studies gene regulation in plants, looking at *cis*-regulatory elements and characterizing transcription factors and miRNA genes and their respective targets, with the objective of understanding how these parts of the plant genome work together in determining spatial and temporal expression of genes. The lab had an important role in the 4-year project to produce a reference genome of maize, providing annotations for the genome and helping to generate a draft haplotype map, a gauge of maize's genetic diversity. They have devoted special attention to examining diversity within maize and grape, aiming to accelerate the development of strategies to introduce new germplasm needed to meet demands of an increasing population and a changing environment. This year, Ware was part of a team that uncovered a surprising degree of genetic diversity in the domesticated grape plant. The lab also has brought fully sequenced genomes into an integrated data framework to enhance the power of their comparative studies. This framework now includes 13 genomes of model plants such as *Arabidopsis* and important agricultural species including maize, rice, sorghum, grape, and poplar.

TRANSCRIPTOMES: VIEW OF GENOME ORGANIZATION AND REGULATION AND FUNCTIONAL ROLES OF NONCODING RNAS

T.R. Gingeras

P. Batut
K. Bell
S. Chakrabortty
C. Davis
A. Dobin

J. Drenkow
D. Fagegaltier
M. Fastuca
S. Jha

I. Lassa
W. Lin
K. Persaud
F. Schlesinger

L.-H. See
H. Wang
C. Xue
C. Zalenski

ENCODE Project

K. Bell, S. Chakrabortty, C. Davis, A. Dobin, J. Drenkow, M. Fastuca, S. Jha, W. Lin, K. Persaud, F. Schlesinger, L.-H. See, H. Wang, C. Xue, C. Zalenski

Overall goals. The goals of the ENCODE Transcriptome Group are to generate a comprehensive collection of maps detailing the sites of RNA transcription and to characterize the diverse collection of RNAs produced at these loci providing transcriptional start sites (TSS), transcriptional termination sites (TTS), RNA splices sites, chemical modifications of RNAs at 5'ends, and the possible product–precursor relationships between mapped long and short RNA transcripts. The Transcriptome Group consists of members from Cold Spring Harbor Laboratory (Hannon), the University of Lausanne (Reymond), the University of Geneva (Antonarakis), RIKEN (Hayashizaki-Carninci), GIS (Ruan-Wei), and Guigo (CRG).

Type of mapped elements. There are nine different classes of RNA elements and two copy-number variation maps for the tier-one cell lines that have been mapped as part of this project. These elements and the cell lines/subcellular compartments that were mapped are presented in Table 1.

Summary of maps generated for 2010. A total of 226 data sets obtained from 113 samples have been submitted to the ENCODE data coordination center (DCC) at the University of California (Santa Cruz) this year. All samples were done in duplicate, and the RNAs described in Table 1 were isolated from whole-cell, nucleus, or cytosolic compartments. For two cell lines studied, the chromatin, nucleolus, and nucleoplasm subcompartments were also analyzed. A detailed dashboard created by our collaborators at CRG in Barcelona of this current year's summary and the summary for the last 3.5 years can be found at http://genome.crg.es/~jlagarde/encode_RNA_dashboard/demo_9.html or at the main ENCODE web site at http://encodewiki.ucsc.edu/EncodeDCC/index.php/Transcriptome_project_%28Gingeras%29.

ModENCODE Project

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

Two studies have been successfully completed this year. The first involves the analysis of the transcriptional output of long RNAs from 25 cell lines identified by the modENCODE consortium as being the common set of control cell lines for the project. *Drosophila melanogaster* cell lines have become valuable resources for developmental geneticists and cell biologists, and their use continues to grow. The analyses are detailed in Cherbas et al. (2010). The results of this analysis indicated that unannotated transcription is substantial (19% of total euchromatic signal in a typical line). Conservatively, we have identified 1405 novel transcribed regions (contigs); 684 of these appear to be novel exons of neighboring, often distant, genes. Of the 14,807 annotated genes probed, 64% were expressed at a detectable level in at least one cell line, but only 21% of genes were detected in all 25 lines. Each cell line, on average, expressed 5885 genes including a core set of 3109 genes expressed in all of the lines. Gene expression levels vary over several orders of magnitude. Major known signaling pathways are well represented

Table 1. Data Types Analyzed in All Samples

RNA types	Total A+ A–
Long (>200 nt)	X X X
Short (<200 nt)	X
CAGE	X X X
PET Ditag	X X
DNA PET	

in the lines and all lines appear to be sensitive to insulin, hedgehog, and BMP (bone morphogenetic protein) signals. Despite variations among lines, most differentiation pathways are "off" and survival and growth pathways are "on." About 50% of the genes expressed by each line are not part of the core set and these show remarkable individuality; 31% are expressed at a higher level in at least one cell line than in any single developmental stage, suggesting that each line is an enriched version of cells that are a small part of the whole organism. This is most strikingly illustrated by the imaginal disc-derived lines where most of the lines can be assigned on the basis of known spatial expression patterns to small regions within the developing imaginal disc. These mappings show remarkable and unexpected stability of even fine-grained spatial determination. As another sign of individuality, no two cell lines are identical in the pattern of transcription factor expression. We conclude that each line, although derived from a mixed cell population, has retained distinctive features of an individual founder cell superimposed on a core "cell line" gene expression pattern. All of the data and expression scores for genes and exons are available from the DCC of modENCODE (http://modencode.org) and also from the websites of the *Drosophila* Genomic Resources Center (https://dgrc.cgb.indiana.edu/) and FlyBase (http://flybase.org/).

The second study involves the transcriptional analyses of 30 developmental time points during the maturation of the *D. melanogaster* embryo to 4-week-old adult (Gravely et al. 2010). These data were combined with analyses of the regulatory elements found in the fly genome and was included as one part of an overall integration manuscript (The modENCODE Consortium 2010). All data cited in this manuscript is available on http://www.modencode.org/.

Role of Antisense RNA as a Global Regulation of Gene Expression

A. Dobin, D. Fagegaltier, I. Lassa

Antisense transcripts are RNA molecules that map in an overlapping fashion to the complementary strand of sense strand transcripts. Their function in regulating gene expression is recognized but is considered to be restricted to a limited number of genes in an organism. In a genome-wide transcriptional analysis of *Staphylococcus aureus,* the use of antisense transcription was observed to enable the processing of sense transcripts by means of creating double-stranded substrates for RNase

III endoribonuclease (I. Lassa et al., in prep.). Removal of RNase III activity uncovers a large but discrete collection of antisense transcripts hidden from detection, as well as allowing for increased levels of the corresponding sense transcripts. These results explain why detection of pervasive antisense transcription has escaped previous detection and provide a mechanism to define the minimum levels of sense RNA expression. Such a mechanism appears to be a common feature of all Gram-positive organisms tested thus far.

Novel Exon Detection by Hierarchical Clustering and Spike-In Controls for RNA-Seq

F. Schlesinger, C. Davis

Within the ENCODE consortium, our lab has generated a collection of more than 120 strand-specific, deeply sequenced RNA-Seq data sets from poly(A)$^+$ and poly(A)$^-$ RNAs from different compartments of several cell lines, including technical and biological replicates and spike-in standards (see ENCODE project described above). These data were tested for incorrect strand assignments of sequencing reads, overamplification, strong sequence biases, mapping problems, and other issues that could affect downstream analysis with the help of external RNA standards (see Spike-In section below). The initial detection of novel expressed elements using both simple coverage analysis and read clustering, considering split-read information about splice sites, was carried out using the element detection algorithm developed for this purpose. The reproducibility of these elements was tested using the irreproducible discovery rate (IDR) analysis (http://www.encodestatistics.org/publications/IDR101.pdf), a novel statistical framework for rank-order correlations of significance values between replicates. In this way, the robustness of algorithm being developed is measured relative to the input noise. This analysis showed a large number of significant, reproducible unannotated expressed elements including many that are antisense and intronic to known transcripts.

Clustering software. A software package for RNA feature detection by read clustering has been developed and implemented. Functional genomic features are detected as clusters of reads from sequencing experiments above local background. They are found by recursively partitioning the genome into blocks of different signals using maximum-likelihood splits, Bayesian model comparison, and a negative binomial error model. This error model

accounts for overdispersion of reads relative to Poisson sampling. This overdispersion can be shown and quantified relative to the known structure and abundance of the external RNA spike-in standards present in our data sets. Using this correct error model is important to prevent detection of spurious significant signal enrichment within features, such as exons and false differences between data sets. In some functional genomics experiments, only the number of reads in a given region is relevant.

In those cases, the complex read-mapping information can be reduced to a cumulative sparse vector of read counts ("signal") along the genome for very fast computation of the signal in any genomic region. However, for the shotgun RNA-Seq, the read alignments themselves contain information, most notably on splicing of transcripts and the exact end of exons. For this purpose, the program was extended to consider reads individually, only counting those falling fully within a given region and considering split-mapping information about splice sites when choosing endpoints of clusters. The tool is multithreaded to exploit the easy parallelism of the recursive clustering problem. All this allows handling and analysis of very large data sets (e.g., >200 million reads of paired-end 76-bp RNA-Seq data) in a relatively short time (depending on the parameters used for analysis) with reasonable memory requirements. In addition to extensive manual inspection on the genome browser, reproducibility analysis suggests that the derived significance values for novel clusters are a meaningful measure of confidence and that the cluster locations and boundaries are consistent between replicates in the presence of biological noise. Further evaluation of the tool will be possible using the results from ENCODE validation RACE (rapid amplification of cDNA ends) experiments of novel intergenic and antisense transcripts currently being performed.

Spike-in controls. Through collaboration with a group at the National Institute of Standards and Technology (NIST), we obtained a pool of 96 reference RNAs with diverse sequence properties and spanning a six order of magnitude range of concentration. Adding them as "spike-in controls" to RNA-Seq libraries primarily allows us to derive a standard curve and convert read counts into absolute RNA concentrations in the studied sample. In this way, robust comparisons between different samples in the absence of internal controls are possible. Additionally, the spike-ins were used to assess the random error and biases of our RNA-Seq protocols to help with reliable downstream analysis, especially the detection of novel features. Fitting different regression models between read counts of each spike-in in different replicate libraries showed the presence of significant overdispersion of noise compared to pure sampling noise. The same can be observed when analyzing variance in the read distribution along each reference RNA. This could be fit by a negative binomial distribution which serves as a robust null model when searching for new features within a sample or for differential expression between samples. They also allowed us to study the sequence-dependent biases in read counts, both within a transcript (where we see strong effects of random hexamer reverse transcription priming) and between transcripts (where overall GC content has the strongest influence). These effects are quite strong and can be included in models analyzing RNA-Seq data. External RNAs make this analysis easier and more robust as they do not suffer from biological variance, alternative isoforms, incomplete annotation, and similar issues. Especially their use in deriving standard curve and error estimates for quantification depending on transcript abundance and sequence features is of general interest (X. Deng et al., in prep.).

Transposons and Evolution of Transcriptional Regulation in the *Drosophila* Clade

P. Batut

Transposable elements (TEs) have long been proposed to have a role in the evolution of gene regulation and have in some particular instances been shown to indeed do so. The overall aim of this project is to study the extent to which transposons have contributed to the evolution of transcriptional regulation in the *Drosophila* group and to characterize some of the mechanisms through which they may have achieved this. Specifically, the role of TE in reshaping the temporal aspects of gene expression throughout embryonic development is an important area to explore. Two mechanisms are being explored through which they may do so: namely, either the contribution of new transcriptional start sites (TSSs) and proximal promoters to existing genes or the addition of new regulatory sequences that affect the activity of existing promoters. A combination of tools comprising experimental and computational genomics is being used to undertake a comparative study of the developmental regulation of transcription by transposons in several species of drosophilids.

Evidence for transposon-derived transcriptional promoters. The massive transcriptome data produced

for *D. melanogaster* by the modENCODE Consortium (The ModENCODE Project Consortium 2010) constitute a rich and structured data set that can be very informative within the scope of this project. These data are being exploited to search for genes whose TSSs lie within transposable elements. The CAGE (cap analysis of gene expression) data set produced from mixed-stage embryos has been searched for TSSs (as defined by CAGE tag clusters) embedded within transposons. This entailed the development of a peak-calling algorithm tailored and optimized for this task. The modENCODE RNA-Seq developmental time course data will now be mined for evidence supporting the existence of transcripts linking such TSSs to annotated genes. Comparative genomics approaches are currently being harnessed to investigate the evolutionary conservation of TSS gene pairs.

Development and optimization of an algorithm for the detection of CAGE tag clusters. Although peak calling is a very common task in genomics, the very unique features of the CAGE data and of the questions we have set out to answer make current algorithms unsuitable for our situation. Thus, an algorithmic approach has been developed that takes into account the particular features of the data (short reads, background signal overdispersion) and of the genomic regions of interest (high repetitiveness) to improve both specificity and sensitivity in the detection of CAGE peaks. This is achieved principally by accounting for missing data at genomic sites where no read can be uniquely mapped. This approach has led to the identification of 23,384 CAGE peaks across the genome, of which 942 fully overlap UCSC Repeat Masker transposon annotations; 700 of these would not have been identifiable without correcting for the mappability of individual genomic positions. After further filtering using multiply-mapping CAGE tags, 687 regions are retained as high-confidence transposon-contained CAGE peaks.

Evidence for transcripts connecting. As a preliminary screen before undertaking this phase, the TE contained TSSs whose genomic position indicates that they may be alternative promoters for annotated transcripts. We have identified 123 such TSSs that lie between the middle point of a Flybase transcript annotation and 15 kb upstream of its 5′ end. The existence of transcripts connecting these TSS gene pairs is now being tested.

Protocol for paired-end Illumina sequencing of 5′-complete cDNAs. It is crucial for the generation of our developmental transcriptome data to make use of an RNA-Seq protocol that both allows for TSS identification and optimizes throughput. An adaptation of CAGE for paired-end sequencing would be ideal for this purpose. Much progress has been made in developing and sequencing paired-end CAGE libraries, optimizing insert size and yield, and minimizing PCR (polymerase chain reaction) amplification. We are now in the process of investigating high-background issues in order to match the high degree of specificity achieved by the Carninci laboratory (RIKEN Institute, Japan), which developed the original protocol. The portion of the protocol that has to be corrected is fortunately identical to this original one, but this troubleshooting will clearly require some more work.

Validation of transposon gene transcript topology by gene-targeted RACE or RT-PCR. A list of candidate transposon-gene pairs has been made using the analysis of the modENCODE data. Reverse transcriptase (RT)-PCRs are being performed to investigate the existence of transcripts linking those genes to their putative transposon-contained TSSs. We are also planning on investigating the evolutionary conservation of functional transposon gene pairs by performing similar RT-PCR experiments for the same genes in other strains of *D. melanogaster*, or for their orthologs in other *Drosophila* species.

RNA-Mediated Intercellular Signaling
S. Chakrabortty

Proper functioning of a multicellular organism requires an intricate and sophisticated system of intercellular communication that ensures proper coordination among different cell and tissue types and synchronization between different cells of the same type. Traditional and well-established modes of intercellular communication include cell–cell contact and cell–ECM (extracellular matrix) contact by gap junctions, secreted signaling molecules and physical interaction of membrane proteins.

Recently, the role of exosomes as communication through intercellular transference of RNA has come into focus. Exosomes as intercellular carriers of RNA and proteins have been appreciated and investigated. Exosomes are small 30–100-nm vesicles of endocytic origin that are secreted to the extracellular milieu on fusion of multivesicular bodies (MVB) to the plasma membrane. Under electron microscopy, exosomes have a character-

istic cup-shaped morphology and have a sucrose density of 1.13–1.19 g/ml. Because of their endocytic origin, exosomes often contain proteins involved in membrane transport and fusion, MVB biogenesis, integrins, tetraspanins, and heat shock proteins. They are also enriched in raft lipids such as cholesterol, sphingolipids, and glycerophospholipids. Upon secretion, exosomes may bind to recipient cells through receptor–ligand interactions, attach and fuse with the target cell membrane, or be internalized through endocytosis. So far, exosomes have been found to be present in almost all biological fluids and secreted in culture by almost all cells investigated. Exosomes from dendritic cells and B cells have been shown to have potent immunostimulatory and antitumor effects in vivo and have been used in antitumor vaccines, whereas those from breast cancer and platelets promote angiogenesis and tumor progression.

Exosomes are an attractive vehicle of RNA transfer because they provide a protected environment thereby ensuring their stability from extracellular RNAs. Exosomes have been shown to carry intact and functional mRNA to neighboring or distant cells that can be translated into proteins upon uptake. microRNA transferred by exosomes can bind and repress its targets in the recipients. Some of the RNA packaged in exosomes are present severalfold higher than the secreting cell, suggesting some kind of sorting mechanism to collect and package specific RNA in the exosomes. Thus, exosome-mediated transport of RNAs may represent an important mode of communication that allows cells to have coordinated intercellular regulation of gene expression.

Isolated exosomal profile. Exosomes are cup or round-shaped membrane-bound vesicles with a floatation density of 1.13–1.19 g/ml. The isolation of these vesicles from cell-free culture media entails three rounds of differential centrifugation. Vesicles are treated with RNase and DNase prior to lysis of the vesicles to evaluate the contents (Fig. 1).

Validation of enrichment of exosomes. Several proteins have been previously identified as part of the inner-membrane component of virtually all exosomes. Tumor susceptibility gene 101 (TSG101) and fibrillarin are two multivesicle body markers that were used to evaluate the enrichment of these isolated exosomes via northern analysis. The results of these analyses showed a 100–1000-fold enrichment. Nucleic acids extracted from the isolated exosomes are sensitive to RNase but
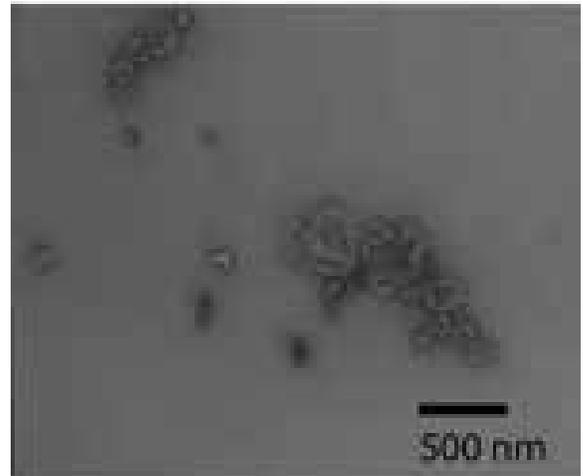
Figure 1. Electron micrographs of isolated exosomes from K562 cell culture medium.

insensitive to DNase. RNase-treated exosomes (prior to lysis and nucleic acid extraction) provide both long (>200 nucleotides) and short (<200 nucleotides) RNAs similar in amount and length to the RNAs isolated directly from exosomes. Both long and short RNAs are being characterized by deep sequencing.

The next stages of this project will include the establishment of primary rat primary neuronal and ganglia cell lines in which each cell type is isolated from a genetically distinguishable strain. The presence of exosomes and the RNA complement present in these exosomes will be analyzed.

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DEVELOPMENT OF NEXT-GENERATION SEQUENCING TECHNOLOGY

W.R. McCombie

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Investigating Genome Variation: Infrastructure Development and Quality Assurance

The Genome Center continued to expand resources for large-scale genomic studies in 2010, in terms of both next-generation sequencing and analysis capabilities and usage by the wider CSHL community. We continued to focus on training and development of standard operating procedures to ensure that quality remained high during this expansion (see Fig. 1).

Four new Illumina HiSeq 2000 instruments were introduced to the Genome Center that increased capacity by fourfold over the Illumina Genome Analyzer IIx (GAIIx) instruments that we replaced. In addition, we upgraded the Illumina software and incorporated an improved enzyme in the reagent kits that allowed for reduced run times.

We also became a beta test site for the new PacBio RS technology that allows for amplification-free single-molecule sequencing. We are focusing on increasing yield and accuracy on this platform, but we have already achieved long read lengths that should complement our shorter Illumina reads.

To address the increased demand for secondary analysis, several postdocs joined the bioinformatics staff, and new alignment and variant detection pipelines were investigated. We will continue to update and augment the informatics workflow in order to provide comprehensive and efficient analyses.

We began working with the Wigler lab on the development of a new LIM system to provide more detailed sample reports, automated status updates, and quality metrics. These improvements will enhance users' ability to monitor and modify the large and diverse projects that are possible with next-generation sequencing.

We have begun the testing of cloud computing and are working with resources such as Galaxy, which provide a simplified web interface to help ease the burden
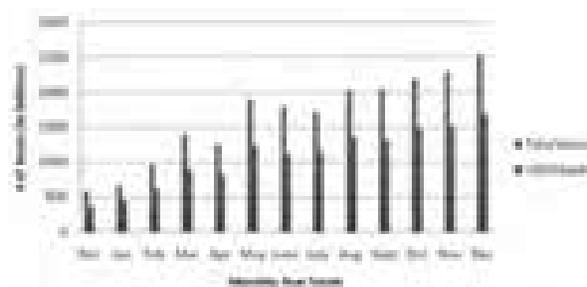
Figure 1. Comparison of total bases generated versus total high-quality bases for monthly Illumina runs from December 2009 through December 2010. We use Q30 bases as a metric to ensure very high data quality.

on local servers, as well as to accommodate users who require assistance with bioinformatic components of their data analysis. Streamlining the analysis pipeline and the tools available to the community will remain an important goal in 2011. This will be done in collaboration with the Bioinformatics Shared Resource of the CSHL Cancer Center (Molly Hammell, Director).

Investigation of Bipolar Disorder Genetics Using Exome Capture and Resequencing

This work was done in collaboration with J. Potash, R. Karchin, and A. Chakravarty (Johns Hopkins School of Medicine) and I. Iossifov (Cold Spring Harbor Laboratory).

Bipolar disorder I (BP) is a major mental illness with a significant genetic component, as demonstrated by family, twin, and adoption studies (Smoller and Finn, *Am J Med Genet C Semin Med Genet 123C:* 48 [2003]). Molecular investigation into the genetics of BP has included linkage analyses and, more recently, several large genome-wide association studies (GWAS) (Wendland

and McMahon 2010). Although GWAS has yielded a small number of genome-wide significant results, the "missing heritability" problem suggests that the majority of variants contributing to BP are rare variants. Other approaches are therefore necessary to uncover these rare BP susceptibility variants. We are applying exome capture and resequencing toward finding rare genetic variants that contribute to BP.

Our work in 2010 involved the exome capture and resequencing of 186 samples, which included a pilot-family-based study, and BP probands and unrelated controls. Each sample was prepared for capture and sequencing using Illumina-compatible sequencing adapters with or without bar codes, individually captured, and sequenced on one lane of an Illumina GAIIx instrument programmed for PE 76 cycle sequencing. Sequence data quality, genome alignment, and exome capture statistics were computed to determine the efficiency of the capture and sequencing using an analysis pipeline developed by Ivan Iossifov. Samples that required more raw sequence data to obtain improved exome coverage were included in a second round of sequencing and pooled during sequencing when possible (with barcode multiplexing).

On average, 36.5 million pass-filter sequence read pairs (5.5 billion bases) were produced per captured sample, with 99.7% of bases attributed to library DNA (rather than sequencing adapter), 93.5% of read pairs properly mapped with unique chromosome start and end coordinates, 57.5% of properly mapped read pairs mapped to exome probe sequences, and 31.3% of the total input pass-filter sequence data mapped to the exome target. The raw sequence data that we produced provided 81.6% of the exome covered at greater than 20x depth, 13,694 coding single-nucleotide polymorphisms (SNPs), 7545 synonymous SNPs, and 6149 nonsynonymous SNPs per sample, on average (Table 1). On the basis of our current exome data, we have identified 275 genes with possibly damaging, nonsynonymous SNPs found in cases but not in controls. There also appears to be an enrichment of nonsynonymous SNPs in BP GWAS-identified genes in cases compared to controls.

Further work for this project will include implementing a custom exome capture design that provides higher coverage of genes that encode postsynaptic proteins; this will allow us to additionally investigate the neurological basis of BP, exome captures with at least ~650 additional cases and ~350 additional controls, and more in-depth analyses of SNPs identified in this project.

Family-Based Analysis of Bipolar Disorder by Whole-Genome Resequencing

This work was done in collaboration with D. Blackwood, K. Evans, and D. Porteous (University of Edinburgh).

We are studying a large Scottish pedigree where many family members have bipolar disorder or recurrent major depressive disorder. A genetic study of this family by our collaborators at the University of Edinburgh suggested that a 20-Mb haplotype on chromosome 4 has significant linkage to bipolar disorder (Le Hellard et al., *Biol Psychiatry 61:* 797 [2007]).

We decided to use next-generation sequencing (Illumina GAIIx) to resequence the entire genome of individuals from this family, focusing analysis on the 20-Mb critical region on chromosome 4. We hope to discover rare variants associated with bipolar disorder susceptibility. Three affected (two with BD, one with MDD) and two unaffected married-in individuals from this family were selected for whole-genome sequencing using paired-end 101- and 125-cycle runs. We achieved 58x–36x coverage on each individual. Reads from all flow cells for each individual sample were then aligned to UCSC hg8. We processed the mapping and SNP calling by using the Burrows–Wheeler alignment (BWA) and sequence alignment map (SAM) tools (Li et al., *Bioinformatics 25:* 2078 [2009]) pipeline. Potential polymerase chain reaction (PCR) duplicates were removed, and error-prone variant calls were then filtered out by using a minimum consensus quality score of 20 and a minimum read depth of

Table 1. Overview of Human Exome Coverage and SNP Calling Results for the 186 Case and Control Samples Captured and Resequenced in 2010

	% Exome target covered at ≥20x	Total no. coding SNPs	No. synonymous SNPs	No. nonsynonymous SNPs
Min	65.43	11,677	6,399	5278
Max	92.56	18,493	10,100	8393
Mean	**81.60**	**13,694**	**8,545**	**6149**

Human exome captures were performed individually with SeqCap EZ Exome Library v1.2 (NimbleGen) and sequenced with paired-end 76-base reads on GAIIx sequencing instruments (Illumina).

Table 2. Number of SNPs Identified by Whole-Genome Sequencing Across the Five Individuals

Individual	Diagnosis	SNPs	Exonic SNPs	Synonymous coding SNPs	Nonsynonymous coding SNPs
Affected individual 1	BD	3,694,870	53,541	11,295	10,827
Affected individual 2	MDD	3,714,782	53,672	11,330	10,863
Affected individual 3	BD	3,619,932	51,836	10,876	10,553
Unaffected individual 1	–	3,573,886	51,635	10,952	10,438
Unaffected individual 2	–	3,594,225	51,155	10,699	10,265

Table lists all SNPs with at least 10x coverage in any one individual.

10x. See Table 2 for the SNP distribution across all five individuals. Each individual has ~3.6 million SNP variants compared to the reference human genome sequence.

Quality control of the sequence data was carried out by comparison of each individual's sequence with previously determined genotype (Illumina IVb whole-genome SNP panel) or exon-based capillary sequence data (for chromosome 4). For affected individuals 1 and 2, the average concordance for each chromosome between genotype data and Illumina sequence data was 94% and 93%, respectively. For affected individuals 3 and 1 and unaffected individual 2, the concordance between the exon-based capillary sequence data and Illumina sequence data for chromosome 4 was 100%, 97%, and 96%, respectively.

We focused our analysis on the 20-Mb disease-linked haplotype (see Table 3 for summary statistics). A total of 292 SNPs were shared by all three affected individuals but were not present in the married-in family members, dbSNP (build 130), or the 1000 Genomes Project (build release date: March 2010).

Table 3 summarizes the data for all SNPs detected on chromosome 4 with at least 10-fold coverage in any one individual. The number in parentheses refers to the number of SNPs that lie outside repeat regions, as defined by using the RepeatMasker track (default settings) from the UCSC Genome Browser (http://genome.ucsc.edu/).

We are currently extending this study to other families that show linkage to the chromosome-4 20-Mb haplotype to genetically refine further the possible bipolar-associated variants. The resulting studies will enhance our understanding of the molecular mechanisms of the cause and development of bipolar disorder; furthermore, these studies will lead to novel diagnoses and treatment of this psychiatric disorder.

Targeted Sequencing of Circulating Tumor Cells from Patients with Castration-Resistant Prostate Cancer

This work was done in collaboration with D. Danilla, C. Sawyer, and H. Scher (Memorial Sloan-Kettering Cancer Center).

Circulating tumor cells (CTCs) are representative of the genomic makeup of a tumor and can provide the molecular snapshot of the disease when treatment is being considered. Somatic mutations detected in CTCs isolated from patients with castration-resistant prostate cancer (CRPC) can serve as predictive markers of tumor sensitivity to targeted therapies. The goal of this project is to identify somatic mutations that are present in CTC, but not in paired white blood cells (WBCs) using next-generation sequencing.

We initialized optimizing sequencing of 75 cancer-related genes for a low number of heterogeneous cancer cells before proceeding to clinical samples. We tested mixes of DNA from LNCaP with DNA from VCaP

Table 3. SNP Discovery by Sequence Analysis of Five Family 22 Individuals

SNPs on chromosome 4	Number
Affected individual 1	267,358 (127,159)
Affected individual 2	271,759 (129,130)
Affected individual 3	261,995 (124,580)
Unaffected individual 1	270,646 (128,372)
Unaffected individual 2	267,699 (127,232)
Subset shared by all three affected individuals	116,034 (56,626)
Subset of above not present in unaffected individuals	30,110 (14,720)
Subset of above not present in dbSNP	3,261 (1,363)
Subset of above not present in 1000 Genomes Project	1,676 (590)
Subset of above that map to 20-Mb linkage region	292 (117)

Genomics   185
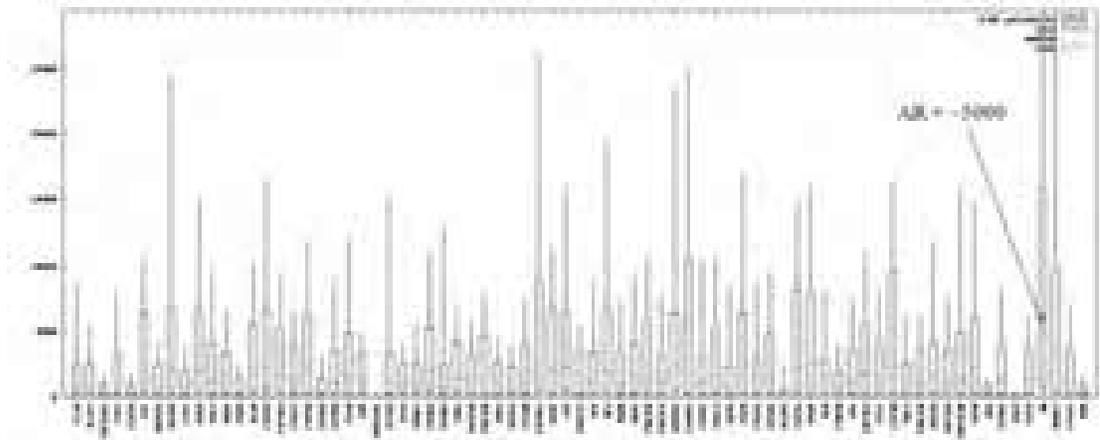
**Figure 2.** Coverage of 75 targeted genes from 50-cell VCaP after WGA. Mean coverage of the amplified AR gene was ~5000x.

prostate cancer cells at 20%, 50%, 80%, and 100% to establish the limit of detection from deep sequencing. We next tested ratio mixes of 50 cells total for isolation and wheat germ agglutinin (WGA). With each set of samples, we performed PCR amplification using a Rain-Dance microfluidic PCR device to stream an emulsion of sample DNA into a microfluidic device that merges that stream with a mix of emulsion droplets containing large numbers of distinct PCR primer pairs. The result is that each reaction occurs individually after the streams merge, because each droplet contains a specific primer pair and eliminates primer competition. The emulsion is used as input for PCR and then broken to isolate the total DNA from the amplification for the gene panel of interest. Sequencing was performed on an Illumina GAII using single-end 36- and 76-cycle runs. Our initial analysis has confirmed the gene panel sequencing coverage (see Fig. 2) and that at least in some cases we can see amplified region amplification by counting the number of reads that match to the amplified region compared to other, nonamplified regions of the genome.

We then performed targeted sequencing on 29 florescence-activated cell sorted (FACS) CTC and WBC whole-genome-amplified samples isolated from eight patients with progressive CRPC. Before sequencing, we also performed PCR amplification using a RainDance microfluidic PCR device on each sample. We identified ~10–100 coding single-nucleotide variants (SNVs) in each CTC sample that were not present in the paired WBC samples. Figure 3 shows a missense amplified region mutation that is present in CTC50 but not in paired WBC20 and WBC10 in a selected patient.

Currently, we are validating selected variants using Sanger sequencing and also extending the study to ad-

ditional patients enrolled in amplified-region-targeted clinical trials using exome sequencing. This study may lead to identification of biomarkers to guide treatment selection for patients with CRPC.

Targeted Resequencing of the Human Disrupted-in-Schizophrenia-1 Gene Using Long-Range PCR and Illumina Sequencing

This work was done in collaboration with D. Blackwood, D. Porteous, P. Thompson, and I. Deary (University of Edinburgh) and P. Visscher and A. McRae (University of Queensland).

Disrupted-In-Schizophrenia-1 (*DISC1*) is a strong candidate gene for bipolar disorder, schizophrenia, and cognitive disorders. A translocation disrupting this novel gene was first described by our collaborators in Edinburgh who showed that it segregated significantly with psychiatric disorder in a Scottish pedigree. In conjunction with our collaborators, we initiated a pilot project to resequence ~523 kb on chromosome 1 containing *DISC1* using long-range PCR. We designed primers to tile across the 0.5-Mb region and showed that we were able to cover up to 98% of our amplified target at >30x coverage with a minimum of 20x needed for accurate mutation discovery. The planned sequencing of ~523 kb of chromosome 1 in 2088 samples was completed during 2009; the data were processed, and putative SNPs were identified using MAQ (Li, *Genome Res 18:* 1851 [2008]). The sample set consisted of 266 bipolar disorder, 277 schizophrenia, 271 major depression, and 1274 control individuals (the majority of controls are

Figure 3. A missense amplified region mutation that is present in CTC50 but not in paired WBC20 and WBC10.

samples from the Lothian Birth Cohort). Validation and analysis of the resulting large number of novel variants are now taking place. Due to the very deep coverage, the analysis parameters for choosing SNPs were a consensus quality of >90 and a read depth of >30X. Variants were screened to exclude repeat regions (RepeatMasker), primer regions, and triallelic regions.

Of the 2088 samples sequenced, 1706 were confirmed by comparison either to available GWAS data or to an SNP typing panel of 12 genotype positions across the region selected by Allan McRae for validation.

We identified almost 4100 variants, with 19% mapping to known polymorphisms (dbSNP130). Interestingly, ~1% (39/4099) were nonsynonymous changes in coding and noncoding exons of *DISC1, DISC2,* or *TSNAX,* which are all genes located in this region. Of these exonic variants, 18% lead to changes in amino acid composition of the encoded protein and potentially its function (Table 4).

Since December 2009, we have been validating these discovered variants using capillary sequencing (ABI 3730). Although our initial validations indicate a high rate of false positives (~50% of selected exonic SNPs were confirmed), our false-negative rate is very low using preexisting genotype data from the majority of

Table 4. Overview of *DISC1* Mutation Screen

DISC1 Summary	
Total confirmed samples	1706
No. with >80% coverage	1552
No. of bipolar disorder	221
No. of schizophrenia	241
No. of unipolar disorder	192
No. of controls + Lothian Birth Cohort	898
Total no. of variants	8267
Total variants after screening	4099
No. of known SNPs (db130)	778
No. in exons inc. untranslated regions	209
No. in *DISC1*	125
No. in *DISC2*	72
No. in *TSNAX*	12
No. in coding regions	56
No. synonomous	17
No. nonsynonomous	39

Descriptive summary of mutation discovery in *DISC1* by resequencing. 1552/1706 (90%) of subjects were sufficiently covered for reliable sequence variant discovery across 80% or more of the target region including *DISC1, DISC2,* and *TSNAX*. 81% of variants were previously unknown. Interestingly, 39/209 (~18%) of all exonic polymorphisms were predicted to be nonsynonymous.

subjects analyzed on alternative technologies. We then tested the individual PCR amplicon pools and found that >80% of the pools showed that the nonreference

allele was detected in the amplicons. Thus, it appears that we introduced a small number of variants into the process (in the long-range PCR) and then effectively detected them. As a follow-up validation, 460 random variants were chosen across the entire region, and ~79% of those variants were confirmed by ABI capillary sequencing. Thus, the rate of validation outside exons appears to be higher, which seems to correlate with findings in the community that evolutionary constraints on exons make variants in those coding regions more likely to be errors due to the presence of fewer real, rare variants. We are continuing with various validation studies to identify the major sources of false positives, including testing alternative alignment software Novoalign (Novocraft), which allows for more sensitive read alignment.

To further investigate our variant calls, our collaborators compared our data to 903 variants (from 60 HapMap samples) that were called in the European-only set of the 1000 Genomes Project (www.1000genomes. org). Minor allele frequency distribution looked quite similar (see Fig. 4), although we called more rare variants, probably due to our greater sequence depth.

We are working on revising our validation plan based on our ongoing results. We plan to complete final analysis of the data this year and publish our findings. It is
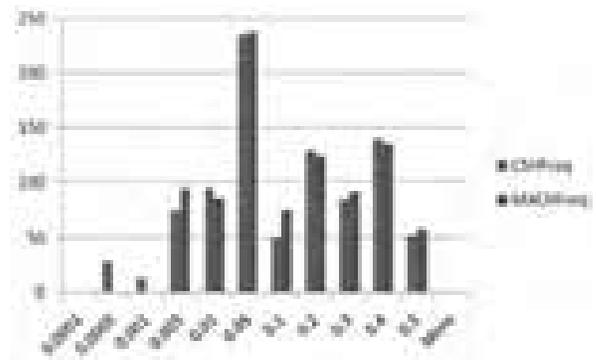
Figure 4. Comparison of minor allele frequency in 903 overlapping variants in the 1000 Genomes (EUR) University of Michigan MACH call set and the CSH DISC1 variant call set.

known that *DISC1* causes cognitive disorders in the initial translocation family. This study will finally determine how frequently other rare mutations in *DISC1* contribute to these disorders and give some perspective on the types of variants that may contribute to disease.

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Rebecca Solomon

GENOME-SCALE DATABASES OF PATHWAYS, GENETIC VARIATIONS, AND EVOLUTION

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          S. Cain   G. Gopinathrao   W. Spooner   Z. Zha
          N. de la Cruz   T. Harris   P. Van Buren   W. Zhao

I moved the bulk of my laboratory to the Ontario Institute for Cancer Research in Toronto, Ontario, in 2009, but I still maintain a small group at CSHL working on the iPlant Collaborative project.

The iPlant Collaborative is a virtual organization of faculty, students, postdoctoral fellows, staff, high-performance computing experts, and professional software developers drawn from CSHL, the University of Arizona, and the Texas Advanced Computer Center. iPLant is designed to solicit and meet biological grand challenge questions from the plant science community, and, in close consultation with this community, create a cyberinfrastructure that makes it possible to meet these challenges. In 2009, two self-forming grand challenge teams were chosen by a community-representative Board of Directors to work with iPlant personnel to develop "Discovery Environment (DE)," a cyberinfrastructure and user interface to help with the biological grand challenges.

Our group works on the iPlant Tree of Life (iPToL) and the Genotype to Phenotype (Gen2Phen) Grand Challenge Project. The fundamental aim of the first project is to assemble a comprehensive phylogenetic tree of half a million known species of green plants for better knowledge of evolutionary relationships, which will yield new insights across the plant sciences. The second project aims to create databases and tools to integrate and analyze experiments that connect the genotype of plants to their physical properties, such as agronomically important traits. Both projects are working toward releasing web-based discovery environments during 2011.

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PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware J.-M. Chia M. Karey Monaco Z. Lu S. Pasternak J. Thomason K. Youens-Clark
A. Chuah S. Kumari C. Noutsos W. Spooner P. Van Buren L. Zhang
A. Eveland Z. Liu A. Olson J. Stein S. Wei

Our lab has dual goals of conducting plant genomics research and of enabling access to genomics resources and discovery tools to the broader research community. Research includes a broad range of activities in physical, statistical, and functional genomics in both model systems and crop plants, with applications in agriculture. During the past year, we were joined by postdoc Christos Noutsos, Marcela Karey Monaco, undergraduate intern Michael Levy, and high school intern Rigina Gallagher. We said good-bye to Liya Ren and Shuly Avraham. On sabbatical from University of Missouri, Columbia, Michael McMullen has joined us as a visiting professor.

Gramene

A. Chuah, M. Karey Monaco, S. Pasternak, W. Spooner, J. Stein, J. Thomason, S. Wei, K. Youens-Clark [in collaboration with E. Buckler, USDA, Cornell University; S. McCouch, Cornell University; P. Jaiswal, Oregon State University]

Gramene was conceived from the notion that the juxtaposition of annotation across multiple genomes by comparative mapping provides a synthesis of knowledge greater than the sum of its parts. Gramene is a collaborative project that leverages sequence and functional information from plant reference genomes to promote research and translational genomics in agriculture. The Gramene website (http://www.gramene. org) serves as a portal to multiple genome browsers and manually curated databases of genes, proteins, biochemical pathways, quantitative trait loci (QTL), ontologies, germplasm, and genetic diversity data. In the last year, our group has completed several milestones on the Gramene project, including its 31st and 32nd releases since 2000. Of the many improvements made, we increased the number of partial and complete sequenced genomes in our Ensembl genome browser to 19 scientifically and economically important species including rice, corn, grape, sorghum, poplar, and the basal land plant Selaginella. Gramene is also home to reference sequences for three cultivated and eight wild species of rice, making it the single most comprehensive genus-level (*Oryza*) resource in plants. This

year, we released pathway databases for maize (MaizeCyc) and *Brachypodium* (BrachyCyc), complementing existing pathway databases for rice, sorghum, and other plants. Gramene is also positioning itself as a principal resource for conducting population-based genetics research. We added several new diversity/phenotype data sets covering *Arabidopsis*, rice, and maize and have added new tools for querying single-nucleotide polymorphisms (SNPs) and visualizing genome-wide association studies. All of this work has contributed to a 38% increase of traffic on the site during the last 2 years from users all over the world. Our databases are created in partnership with the European Bioinformatics Institute (EBI) and are now also available through the Ensembl Plant portal. During the next year, we will continue to add new reference genomes, data sets, and functionalities to this resource.

Maize Project

S. Pasternak, A. Olson, J. Stein [in collaboration with the Maize Genome Sequencing Consortium; J. Glaubitz, Cornell University; E. Buckler, USDA, Cornell University; M. McMullen, USDA, University of Missouri, Columbia]

Our group leads data coordination, analysis, and visualization for the Maize Genome Sequencing Consortium, a 4-year, multi-institutional project funded by the National Science Foundation (NSF). In the last year, we released version 5b.60 of MaizeSequence.org, the official public portal of the maize project. This release is based on the most recent version of the reference assembly (B73 RefGen_v2) for which we conducted a new evidence-based gene build. The new gene set includes improved gene models for thousands of loci and hundreds of new high-confidence loci that were not detected in the previous version of the assembly. Despite the many improvements to the reference sequence, there are still gaps of missing genes and stretches of sequences that are not definitively positioned within chromosomes. In a new initiative, we are working to capture missing gene space using de novo assemblies of whole-genome shotgun sequence reads. We have devel-

oped a novel approach that builds gene-centered scaffolds by guided assembly onto cDNA sequences. To anchor these, we are taking advantage of a very high-resolution genetic map containing nearly half a million markers generated by a genotype by sequencing approach. Preliminary data indicate that we are able to anchor thousands of de novo contigs corresponding to hundreds of genes. These data will contribute to future improved assemblies of the maize reference genome.

Maize HapMap Project

J.-M. Chia [in collaboration with E. Buckler, USDA, Cornell University; M. McMullen, USDA, University of Missouri, Columbia]

Maize has abundant diversity that is evident from the large variation of observable traits in the species. This diversity translates to the DNA level, where large differences exist between individuals of the species. Much of this genetic diversity in maize resolves to single DNA base differences (SNPs), but perhaps more significantly, structural variations as well as copy-number variations have a strong impact in the genetic diversity in the species. As a result, the concept of a maize genome is fluid, with DNA content varying greatly between individuals of the species. It is estimated that 30% of the genome of each maize inbred line is unique to itself and this unique space contains genes, thus likely to impact traits.

With this in mind, we set out to study the genetic variation in the species. In this iteration of the Maize HapMap Project, using high-throughput DNA sequencing technologies, we scale out the breadth and depth of the study by resequencing more than 100 inbred lines of the *Zea mays* genus. The volume of sequence generated per inbred line is approximately five times the size of the genome, and from these sequences, we can identify variant loci. Given the complexity of the genome, these variations were scored using carefully designed population genetics-based filters that leverage the carefully constructed maize breeding populations. In total, more than 55 million SNPs were identified. Furthermore, by comparing the distribution of read depth across the genome we could determine copy-number and structural variations. The results reveal the plasticity of the genome with more than 80% of the genome affected by copy-number or structural variation. We used the combined set of SNPs and structural variations as markers and tested the potential of these to affect maize traits. The results highlight the signifi-

cant impact of structural variation on traits. Finally, because wild ancestors of domesticated maize were included in the sampling population, based on regions of lower genetic diversity, we are able to address questions about genes that were selected for during the domestication and improvement of maize as a crop.

Maize breeders have long been exploiting the diversity in maize for improving the yield of this crop through selective breeding programs, contributing to its current position at the apex of the production crop list. We are in an era where traditional crop-breeding techniques are being supplemented and, in some cases, supplanted by molecular breeding approaches that speed up the breeding process. Understanding the diversity in maize and how it impacts agronomic traits is crucial in implementing these new approaches and will be an important platform for sustainable agriculture in the future.

The iPlant Collaborative

J. Lu, C. Noutsos

This project employs more than 50 staff and is headquartered at the University of Arizona (principal investigator Stephen Goff). Dozens of collaborators hail from over 20 institutions.

Driven by the large-scale generation of data, ranging from genomics to imaging, research in biology is becoming increasingly information-based. Lagging behind is the ability of individuals to make efficient use of this information because of limitations in data access, computational infrastructure, and available tools. In addition, to tackle big questions in biology, researchers need to collaborate in effective ways to reach across institutional and disciplinary lines. The iPlant Collaborative (http://iplant collaborative.org) is an NSF-funded cyberinfrastructure project that provides access to high-performance computing, data storage, and tools via customized "Discovery Environments." The project's mission is to foster the development of a diverse, multidisciplinary community of scientists, teachers, and students who will apply transformative approaches and computational thinking to address grand-challenge questions in plant biology. In its first 3 years, iPlant has engaged the plant science community to develop two Grand Challenge projects: Genotype to Phenotype (iPG2P) and the Tree of Life (iPToL).

The goal of the iPToL Grand Challenge is to assemble a comprehensive phylogenetic tree that describes the diversification and evolutionary histories of the half-million described species of green plants. The sheer scale of this endeavor will require a 100-fold scale-up

of existing phylogenetic reconstruction methods and concomitant innovations in tree visualization and post-analysis. One fundamental requirement is to start with standardized taxonomic names for each species—a non-trivial task. Erroneous and synonymous taxonomic names are a major challenge for virtually every field of plant biology. Large organismal databases (GBIF, SpeciesLink, VegBank, SALVIAS, TraitNet, GenBank, TreeBASE) are plagued by taxonomic error and uncertainty. In some databases, up to 30% of names do not match to any published name. Furthermore, 5%–20% of published names may be synonymous. Currently, correcting and harmonizing taxonomy are the time-consuming and ad hoc responsibility of the individual researcher. Thus, a subproject of iPToL is development of iPlant's Taxonomic Name Resolution Service (TNRS). Creating this TNRS tool will remove a barrier scientists must now overcome to conduct higher-quality comparative biodiversity science. With its deployment in iPlant's Discovery Environment, TNRS will be available to others wishing to perform similar taxonomic name resolutions on their data sets, enabling a wider community to expand the public scientific knowledge base. The TNRS is currently in beta-testing phase.

The iPG2P project aims to elucidate the relationship between plant genotypes and the resultant phenotypes in complex environments, one of the foremost challenges in biology. We contributed efforts to incorporate ultra-high-throughput sequence (UHTS) data analysis work flows into the Discovery Environment. The work flows enable users to work with data from Illumina and AB SOLiD sequencing machines to (1) preprocess UHTS sequence data, (2) determine transcript abundance (RNA-Seq), and (3) perform variant detection analysis. This will allow the general research community to access UHTS technology.

The Maize Methylome Project

J. Lu [in collaboration with A. Rafalski, DuPont Crop Genetics; R. Martienssen, Cold Spring Harbor Laboratory]

Heritable differences between maize germplasms can be caused by epigenetic loci that affect gene function by modification of chromatin structure. One type of modification, DNA methylation, has an important role in the regulation of gene expression and control of transposable elements. To begin to understand how variations in methylation affect phenotype, we have sequenced the methylomes of two heterotic maize inbreds, B73 and Mo17. The method used bisulfite to convert unmethyl-

ated cytosine to thymine, followed by Illumina sequencing to generate paired-end reads. For each background, we have generated 20X–30X coverage over the mappable portion of the maize genome. This has revealed hundreds of millions of cytosine methylation sites mapped with single-base resolution. Preliminary analysis shows that patterns of methylation correlate closely with annotated genome features, such as genes, expressed regions, and repeats. Future work will pinpoint the differences between the two inbreds to provide a basis for investigation of how these differences affect gene regulation and other biological functions.

A Systems Approach to Infer Developmental Networks in Maize

A.L. Eveland, S. Kumari [in collaboration with D. Jackson, Cold Spring Harbor Laboratory; M. Beatty, H. Sakai, DuPont Crop Genetics]

The integration of various genome-scale data types can reveal biologically significant information at the systems level that may otherwise not be accessible from analysis of a single data set. Recently, the availability of a maize reference genome and next-generation genomics tools has promoted maize as a central model for grain yield and bioenergy. In this work, we evaluated methods for analyzing sequence-based expression data with respect to the maize genome, as well as in comparative analyses with other plant genomes. We developed analysis pipelines, which include various computational methods to interrogate large data sets and extract relevant metadata in the context of heterogeneous genomic information. These analyses serve as a baseline for inferring networks underlying maize developmental processes and response to environment. Here, we initially focused on a system for maize inflorescence development and utilized RNA-Seq libraries and predicted *cis*-regulatory modules in the promoter region of maize inflorescence genes based on the position weight matrices (PWM).

A primary objective in 2010 was to establish robust methods for analysis of sequence-based expression data and for deriving biological significance related to maize developmental processes. Our RNA-Seq pipeline includes data processing, mapping sequence reads to a reference genome, quantifying expression of a given locus, and using statistical methods to test for differential expression and to cluster genes with similar expression signatures. An initial study was published based on our maize inflorescence development model, which de-

scribed methods for analysis of sequence-based transcriptome profiling in maize and identified candidate genes involved in regulating stem cell fate in maize ear primordia (Eveland et al. 2010). In this study, we used comparative genomics to leverage information from other plant genomes in the analysis of uncharacterized maize genes. We identified ~850 genes that showed altered expression profiles upon perturbation (loss-of-function mutation) of a key developmental regulator in 2-mm maize ear primordia. Among these, specific classes of transcription factors (TFs), such as AP2-EREBP family members, were coexpressed. Interestingly, AP2-EREBP genes have been implicated not only in developmental processes, but in stress-based networks as well. This work also highlighted the potential impact of antisense transcription in our analysis of gene expression data in maize. Approximately 49% of genes showed clear evidence for both sense and antisense expression and ~200 genes showed a twofold change in sense versus antisense transcription in the mutant background.

We also used the available RNA-Seq data sets to validate annotated transcript models and to uncover potentially novel transcripts. The current data sets show significant differences in levels of transcript variants associated with developmental stage and tissue specificity. We used a clustering strategy to identify genes that shared similar expression profiles during development in wild-type inflorescences and characterized classes of TFs with specific spatiotemporal expression signatures and determined whether their expression patterns were altered upon genetic perturbation. We also determined whether clusters of coexpressed genes shared common *cis*-regulatory elements in proximity to their annotated transcription start sites (TSS).

Characterization and Conservation Studies of the *Arabidopsis stele* Gene Regulatory Network

L. Zhang, S. Kumari [in collaboration with S. Brady, University of California, Davis]

Tightly controlled gene expression is a hallmark of multicellular development and is accomplished by TFs and microRNAs (miRNAs). We are using high-throughput yeast one-hybrid (Y1H) and yeast two-hybrid (Y2H) assays to map the root *stele*-enriched TF and miRNA gene regulatory network (GRN). We also integrated computationally identified miRNA–mRNA interactions and the analysis resulted in a GRN composed of 103 interactions between 58 TFs and eight miRNAs (Fig. 1). The strength of many of these interactions was experimentally quantified using systematic perturbation and modeling. Although root or vascular phenotypes were associated with only 16% of these TFs, we identified molecular or gene expression phenotypes for 65%. This indicates that gene redundancy does not solely occur at the level of gene regulation and that gene expression changes may be canalized or buffered. TF and miRNA promoters with the most interactors corresponded to key developmental regulators. Recent work has focused on improving and extending the existing TF library and development of an automated enhanced Y1H (eY1H) mating pipeline, which has been validated. Using this system, we increased the number of interactions with these promoters from 33 to 82 and have now identified interactions with all 13 promoters.

Our next goal is to identify the critical TF–promoter interactions with conserved function in different species.
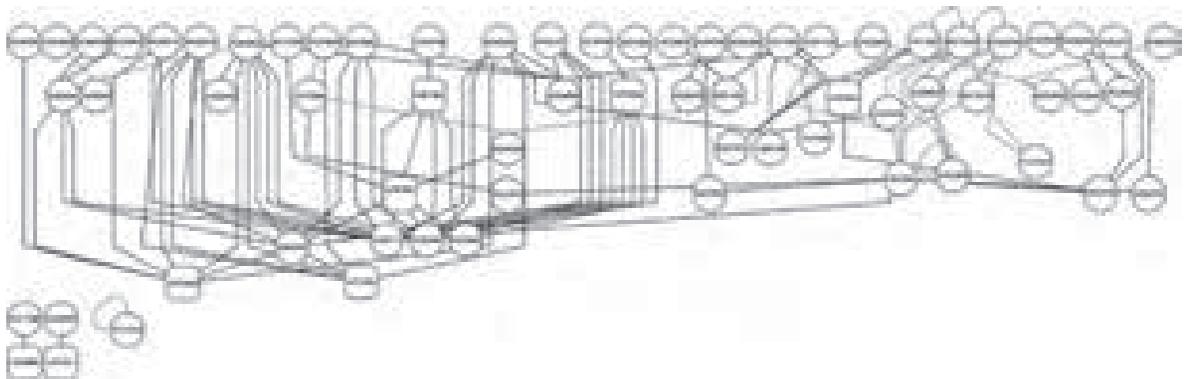

Figure 1. The *stele*-enriched gene regulatory network depicting 103 interactions among transcription factors, promoters, miRNAs, and miRNA targets. (From Brady et al. 2011.)

We projected sorghum orthologs onto the *Arabidopsis stele* GRN to identify the conserved macromolecular interactions. The identification of putative conserved interactions can be used to model the network in different species to determine if these interactions are evolutionarily conserved and to generate testable hypothesis.

Dissection of the Regulatory Network Underlying Waterlogging Stress in Maize

Z. Liu [in collaboration with Professor Y. Zheng, Huazhong Agricultural University, China]

Waterlogging is a critical factor constraining maize production in tropical and subtropical regions. In southeastern China and other parts of the world, excessive springtime rainfall inhibits seed germination and severely damages growing seedlings, leading to yield losses of up to 30%. The primary effect of water submergence is the lack of available oxygen to roots. Survival depends on the degree to which plants are able to mount a myriad of adaptive responses ranging from metabolic switching to complex developmental changes. We have devised an experimental system to monitor stress responses in growth chamber reared seedlings that takes advantage of natural variation among maize inbreds: Hz32 (tolerant line), B73 (mid-tolerant line), and Mo17 (sensitive line). On the basis of the sequencing of small RNA libraries, we identified 21 miRNA genes that have significant changes in expression in the Hz32 tolerant line after 4 hours of treatment. Using quantitative assays in a time-course experiment, we found dramatic differences between the inbred lines with respect to miRNA expression profiles (Fig. 2). In the sensitive line, miRNAs were generally repressed or showed late, weak, or transient induction. This contrasts with the tolerant line in which miRNAs generally showed early, strong, and persistent induction. The mid-tolerant line exhibited a distinct pattern in which some miRNA genes were induced early and persistently and others were not induced or repressed. These differences suggest that miRNA genes have a role in pathways connected to the ability of plants to survive waterlogging stress. Clustering of the miRNA expression profiles suggests that groups of miRNA genes may be coregulated within common gene regulatory networks. In future work, we plan to analyze miRNA promoters to detect potential *cis*-regulatory modules that may explain expression patterns. We also plan to profile transcripts of TF genes that are predicted to be targets of miRNAs.
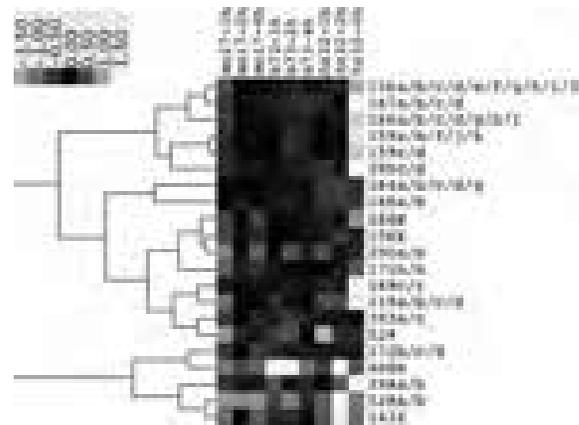
Figure 2. Expression profiles of 21 miRNA genes after waterlogging treatment in a sensitive line (Mo17), mid-tolerant line (B73) and a tolerant line (Hz32). Chamber-grown seedlings were submerged in water up to the soil line at time 0 and root samples were taken at 1, 2, and 4 hours. Transcripts were assayed by quantitative PCR. Fold changes are shown, averaged from three biological replicates, relative to untreated controls.

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QUANTITATIVE BIOLOGY

CSHL recently opened the Simons Center for Quantitative Biology (SCQB). The areas of expertise in the SCQB include applied mathematics, computer science, theoretical physics, and engineering. Members of the SCQB interact closely with other CSHL researchers and apply their approaches to research areas including genomic analysis, population genetics, neurobiology, evolutionary biology, and signal and image processing.

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

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

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 short sequencing reads. He is a pioneer in applying the power of many computers at once to solve data-intensive biological problems—often called "cloud computing." Schatz has developed several breakthrough applications under the National Science Foundation (NSF) cloud computing initiative, and he brings that expertise to harness the collective power of the thousands of computing cores on the CSHL campus to genomics, particularly within the CSHL Cancer Center and the plant biology group. In 2009, Schatz published the first cloud-based sequence analysis tool, called Cloudburst, which dramatically accelerates mapping short sequencing reads to a reference genome with applications to genotyping and expression profiling. Since then, he has published a series of high-throughput algorithms using this technology for single-nucleotide polymorphism (SNP) profiling, sequencing error correction, machine learning, and other important applications. He is currently developing a cloud-computing-based genome assembler called Contrail and a suite of large-scale comparative genomics tools that he plans to use in a collaborative project with the Wigler lab to identify de novo mutations within ~3000 families in which only one child is affected with autism.

Michael Zhang's laboratory develops mathematical and computational methods that can be combined with advanced experimental technologies to transform data into biological knowledge about transcription and gene expression, work that has manyfold implications for the study of cancer and

other diseases. Their tools, used by investigators throughout CSHL and beyond, are designed to identify functional genetic elements within molecular sequences as well as pathways that control and regulate gene expression. Zhang's group has developed a series of computational tools that make use of statistical pattern-recognition techniques to identify exons, promoters, and posttranslational modification signals in large genomic DNA sequences. They also study alternative splicing of exons and collaborate with other labs to characterize splicing enhancers and silencers.

QUANTITATIVE BIOLOGY

G.S. Atwal   R. Aboukhalil   S. Shringapure
                  Y. Cai   J. Weiss
                  B. Fendler

Suyash Shringapure, a summer research intern during 2009, has returned to Carnegie Mellon University to resume his graduate studies at the computer science department, but he will continue to collaborate with Bud Mishra and myself on the project we initiated at CSHL. Joshua Weiss completed work as a volunteer as a member of the Partners for the Future program.

Genome-Wide Interchromosomal Interactions and Population Differentiation

Physically interacting proteins or subunits of proteins are expected to evolve in a correlated manner in order to preserve the functional interactions between them. The sequence changes accumulated during the evolution of one of the interacting units can be compensated by changes in the other. Recent studies have shown that strong pairwise correlations exist between amino acid residues of the same protein necessitated by compensatory mutations. Furthermore, we previously showed that functional single-nucleotide polymorphisms (SNPs) in different genes in the p53 network are correlated across different human populations, arguably to maintain homeostatic balance of p53 activity. We performed a genome-wide analysis of all the interchromosomal pairwise interactions of exonic SNPs in all 11 Hapmap populations. We find extensive evidence of significant interactions, backed up by an analysis of the similarity of protein functions. Protein network analysis demonstrated that correlated proteins were separated by significantly fewer nodes in the protein–protein interaction network. Finally, we showed that correlated SNPs exhibit greater population differentiation, indicating that local geographic adaptation in humans is dependent on networks of correlated polymorphisms rather than simply independent mutations.

Cancer Risk and Haplotype Structure of the TSC1 and TSC2 Genes

TSC1 acts coordinately with *TSC2* in a complex to inhibit mTOR, an emerging therapeutic target and known promoter of cell growth and cell cycle progression. Perturbation of the mTOR (mammalian target of rapamycin) pathway, through abnormal expression or function of pathway genes, could lead to tumorigenesis. *TSC1* and *TSC2* expression is reduced in invasive breast cancer as compared with normal mammary epithelium. In a collaborative effort with the Cancer Institute of New Jersey, association studies were performed on *TSC1* and *TSC2* SNPs for their associations with clinical features of breast cancer. *TSC1* and *TSC2* haplotypes were constructed from genotyping of multiple loci in both genes in healthy volunteers. SNPs were selected for further study using a bioinformatics approach based on SNP associations with drug response in NCI-60 cell lines and evidence of selection bias based on haplotype frequencies. Genotypings for five *TSC1* and one *TSC2* loci were performed on genomic DNA from 1137 women with breast cancer. This study found that for *TSC1* rs7874234, TT variant carriers had a 9-year later age at diagnosis of estrogen-receptor positive (ER+), but not ER−, ductal carcinomas ($P = 0.0049$). No other SNP locus showed an association with age at diagnosis, nor any other breast cancer phenotype. *TSC1* rs7874234 is hypothesized to be functional in ER+ breast cancer because the T allele, but not the C allele, may create an ER element site, resulting in increased *TSC1* transcription and subsequent inhibition of mTOR.

Regulation of Human Fertility by SNPs in the p53 Pathway

We continued our work in collaboration with Cornell Medical School and the Cancer Institute of New Jersey, investigating the association of human fertility and genetic polymorphisms in the p53 pathway. The tumor suppressor protein p53 has an important role in maternal reproduction in mice through transcriptional regulation of leukemia inhibitory factor (LIF), a cytokine crucial for blastocyst implantation. To determine whether these observations could be extended to humans, a list of SNPs in the p53 pathway that can modify the function of p53

was assembled and used to study their impact on human fertility. Selected alleles in SNPs in *LIF*, *Mdm2*, *Mdm4*, and *Hausp* genes, each of which regulates p53 levels in cells, was found to be enriched in IVF (in vitro fertilization) patients. More recently, we have begun to investigate the association of SNPs in p63 and p73 and the initial results are encouraging, although we await an increase in sample numbers for the results can be deemed to be statistically significant.

Fine-Scale Detection of Recent Selection Signatures Using Haplotype Entropy

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

Localization of Tumor Suppressor Genes

Preliminary results from the Lowe Lab's ongoing efforts to identify new tumor suppressor genes (TSGs) by in vivo screens in mice have found instances of tumor suppressor genes in close proximity to each other. We carried out a systematic analysis of the spatial distribution of known (TSGs) to test the hypothesis that there exists an evolutionary selection pressure to cluster TSGs throughout the genome. To this end, we developed a computational and analytical model to determine the extent to which we expect to find adjacent TSGs under no selection. Preliminary results indicate a strong tendency for tumor suppressor genes to cluster and for this clustering to occur across many species. Furthermore, by analyzing genome-wide microarray data, we show that proximal TSGs exhibit significant correlation in their expressions. Ongoing investigations are focused on understanding the implications of this spatial distribution of TSGs for the risk and development of human cancer.

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IN SILICO CANCER GENOMICS

A. Krasnitz   M. Kato
             G. Sun

Research in our group is focused on in silico cancer genomics. In the last 5 years, there has been explosive growth in the volume as well as quality and detail of cancer-related genomic data available for analysis. This development came about through rapid accumulation of public data sets generated by individual laboratories worldwide, through large-scale cancer genome projects such as The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC), and with the advent of new experimental methodologies, especially next-generation sequencing. We see our goal in channeling this flood of data into a number of clinically relevant applications, including pinpointing and prioritizing targets for functional analysis, discovery of genomic markers for clinical outcome, and molecular classification of cancer. All our work is done in close coordination with experimental studies performed by the Wigler, Powers, Lowe, and Stillman laboratories at CSHL.

Recurrence and Search for Drivers in Cancer Genomes

Evidence suggests that recurrent somatic genomic aberrations in cancer are at least partially due to selective pressure on regions harboring oncogenes and tumor suppressor genes. Conversely, one can expect regions of recurrent aberration to be enriched in such driver genes. Recurrence is particularly pronounced in cancer-related somatic copy-number variation. We set out to build computational tools exploiting this property of cancer genomes and make optimal use of both in-house and public DNA copy-number data in order to derive, for each cancer type considered, a set of regions of recurrent and consistent copy-number variation and to compile prioritized lists of candidate driver genes. Starting with genomic profiles of individual tumors, we sift through putative copy-number events and eliminate experimental errors. Next, we mask germline mutations and infer, for any given genomic location, how many copy-number changes it has undergone. Finally, we round event boundaries to the nearest gene affected by the event.

The resulting set of preprocessed genomic profiles

serves as input for our multigenome analysis. Several versions of the latter exist. In the simplest of these, each copy-number event is interpreted as evidence that genes affected by the event are under selective pressure. We further assume that this evidence is shared equally among all of the genes affected by the event. For each gene, its share of evidence is then summed over all events of a given sign (gains or losses) to result in a score prioritizing the gene as a candidate oncogene or tumor suppressor. In early 2010, we applied this technique to a set of ~240 DNA copy-number profiles of serous ovarian tumor tissues, collected and analyzed by TCGA using Agilent 244A array-CGH (comparative genome hybridization) platform. Our findings formed the bulk of an oncogene candidate list of 105 genes. Oncogenic potential of these genes was subsequently examined by Scott Powers' lab in a murine ovarian cell line; 35% of the candidates were found to induce oncogenic transformation upon overexpression.

An alternative strategy consists of deriving a set of genomic regions subject to recurrent and consistent copy-number variation. For a region contained in an event, we say that the region "explains" its portion of the event. The algorithm first finds a region that gives maximal explanation of the data and proceeds iteratively finding, at each step, the most explanatory region for the data not explained so far. This procedure, complete with statistical analysis of significance and reproducibility of findings, is dubbed FOCAL (finder of consistently aberrant loci). The benefit of this analysis is twofold. First, regions delineated by FOCAL are expected to be enriched in cancer-related genetic elements. Second, a drastic reduction of complexity is achieved: from between 105 and 106 CGH array probe readings per tumor to a much smaller (dozens or hundreds) set of observables, each indicating the degree to which a FOCAL region is present in a tumor.

FOCAL has found a number of applications in the course of 2010. A set of ~230 DNA copy-number profiles of advanced breast tumors of patients in two CALGB (cancer and leukemia group B) clinical trials was generated by Dr. James Hicks at CSHL. The data from the two trials were combined in order to examine

clinical response to trastuzumab. Although trastuzumab is routinely prescribed to patients with an amplification of *ERBB2* gene, we identified two FOCAL regions as potential markers for benefit from trastuzumab in patients with no such amplification. This finding suggests the possibility of significantly improving the overall survival in ~15% of advanced breast cancer cases.

In collaboration with Dr. Kandasamy Ravi of the Wigler lab, we applied FOCAL to genomic profiles of mammary gland tumors in mice with a variety of genetic backgrounds. Here again, we observed that reduction of the profiles to strings of values at FOCAL regions retains enough biological information to robustly classify the tumors by their genetic backgrounds (TP53 loss, TP53 and PTEN loss, TP53 and BRCA1 loss, BRCA1 loss, point mutation of BRCA1).

Finally, we used FOCAL to analyze a massive (516 profiles) high-resolution (Agilent 1.1 million probe CGH platform) DNA copy-number data set accumulated by TCGA for serous ovarian cancer and used the output for molecular subtyping of the disease.

Statistically Supported Tree-Based Clustering and Molecular Classification of Cancer

Tree-based clustering has long been an exploratory tool of choice for unsupervised interpretation of complex biological data, and it has found a broad range of applications, from fossil-driven reconstruction of phylogenies to molecular subtyping of cancer. In our recent study, we sought to substantially boost the utility of this technique by assigning statistical significance to the branches of the tree and thereby enabling the researcher to identify statistically meaningful subsets of the data. The validity of this novel approach does not depend on

the form of dissimilarity between data items or on the agglomeration rule for growing the tree. We tested this technique in a number of contexts. In application to a published set of expression profiles of breast cancer, we found that established molecular subtypes of the disease correspond to statistically significant branches of a tree grown from the set. Likewise, application of the method to a set of sequence-based copy-number profiles of 100 individual cells harvested from a breast tumor resulted in a four-way partition of the set corresponding to the four major cell populations found in the tumor and differing from each other by overall amount of DNA in the nucleus.

Next, we used statistically supported clustering to partition the TCGA set of 516 genomic profiles into four classes. Our preliminary analysis suggests that there are significant genomic and clinical differences among the classes in terms of tumor grade, disease-free survival, and point mutation frequency. To our knowledge, this may be a first example of molecular subtyping of cancer based on genomic data, rather than transcriptional data.

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COMPUTATIONAL GENOMICS

M.C. Schatz   M. Titmus

I joined CSHL in July from the Center for Bioinformatics and Computational Biology at University of Maryland College Park. Previously, I participated in the genome assembly and comparative genomics group at the Institute for Genomic Research (TIGR, now the J. Craig Venter Institute) and in the Ravel Lab for Microbial Genomics at the University of Maryland School of Medicine. Since joining the lab, I have recruited Matthew Titmus from the Molecular and Cell Biology graduate program at Stony Brook University.

Cloud Computing and the DNA Data Race

In the race between DNA sequencing throughput and computer speed, sequencing is winning by a mile. Sequencing throughput is currently ~200–300 billions of bases per run on a single sequencing machine, and it is improving at a rate of about fivefold per year. In comparison, computer performance generally follows "Moore's Law," doubling only every 18 or 24 months. As the gap in performance widens, the question of how to design higher-throughput analysis pipelines becomes crucial. One option is to enhance and refine the algorithms to make better use of a fixed amount of computing power. Unfortunately, algorithmic breakthroughs of this kind, like scientific breakthroughs, are difficult to plan or foresee. The most practical option is to develop methods that make better use of multiple computers and processors in parallel.

Parallel computing systems have existed for decades in different forms, but a new system called MapReduce is especially promising for accelerating data intensive genomics research. MapReduce was developed at Google for their large data analysis, especially for scanning across billions of webpages to find the most relevant pages for a search query. Google currently uses MapReduce to analyze ~1 exabyte of data every month. The power of MapReduce is that it can intelligently distribute computation across a cluster with hundreds or thousands of computers, each analyzing a portion of the data set stored locally on the compute node. After an initial round of independent parallel computations, the ma-chines efficiently exchange intermediate results, from which the final results are computed in parallel. The system was originally developed for text and Web processing and was only available at Google, but an open source implementation called Hadoop is now available to install on any cluster for any application domain.

The first-ever application of this technology to genomics, which I in published 2009, was to accelerate the short-read mapping algorithm CloudBurst. In CloudBurst, many computers work together using Hadoop to map reads to a reference genome allowing for any number of differences to determine from where each read originated and if any variations are present. In experiments with 96 cores, the mapping algorithm was ~100 times faster than using a single core, thus reducing the runtime from several weeks to a few hours. The compute cluster used for these experiments was completely virtual and was leased from the Amazon Elastic Compute Cloud (EC2), which offers computing time in their cloud at a small fee per node per hour used.

In a follow-up system called Crossbow, I partnered with colleagues at the University of Maryland to extend the design of CloudBurst to further enable high-performance genotyping. Crossbow identified 3.2 million single-nucleotide polymorphisms (SNPs) from 2.7 billion short reads comprising 38X coverage of the human genome. Using Hadoop to distribute the computation across 320 computer cores rented from Amazon EC2, Crossbow completed the analysis in less than 4 hours whereas it would have taken >1000 hours on a single computer.

Since these pioneering efforts, numerous applications have been developed to leverage Hadoop and cloud computing. For example, in the last year, I and colleagues developed a system called Quake to detect and correct errors in DNA-sequencing reads. Quake is now a standard component of several genome assembly and analysis pipelines, and it uses a maximum likelihood approach incorporating quality values and nucleotide-specific miscall rates to pinpoint and accurately correct errors. By using Hadoop, Quake can scale to virtually any sized genome, including correcting more than 1 billion reads from a deep coverage human genome resequencing study. In a second project, I de-

veloped a Hadoop-based framework called Schimmy for accelerating an entire class of analysis problems related to graphs. Graphs themselves are analyzed in many important contexts, including module detection of protein–protein interaction networks, and genome assembly. Many graphs of interest are difficult to analyze because of their large size, often spanning millions of vertices and billions of edges. Schimmy optimizes Hadoop for large-scale graph analysis, and our experiments demonstrate how Schimmy reduces the runtime of an important machine-learning graph algorithm called PageRank on a graph with 1.4 billion edges by nearly 70%. Future work for my lab is to develop additional high-throughput sequence analysis pipelines using Hadoop and other enabling high-performance technologies.

Genome Assembly

A second major research focus of my Lab is developing enhanced methods for de novo genome assembly and applying those methods to reconstruct the genomes of previously unsequenced organisms. Second-generation sequencing technology can now be used to sequence the equivalent of an entire human genome in a matter of days and at low cost. Sequence read lengths, initially very short, have rapidly increased since the technology first appeared, and we now are seeing a growing number of efforts to sequence large genomes de novo from these short reads.

de Bruijn graphs are a theoretical framework underlying several modern genome assembly programs, especially those that deal with very short reads. In a study published last year, I and colleagues described a novel application of de Bruijn graphs to analyze the global repeat structure of prokaryotic genomes. This study was the first survey of the repeat structure of a large number of genomes, and it gives an upper-bound on the performance of genome assemblers for de novo reconstruction of genomes across a wide range of read lengths. This work demonstrated that the majority of genes in prokaryotic genomes can be reconstructed uniquely using very short reads even if the genomes themselves cannot. In addition, the nonreconstructible genes are overwhelmingly related to mobile elements (transposons, IS elements, and prophages).

In a major application of genome assembly, I contributed to a substantial effort to sequence the domestic turkey (*Meleagris gallopavo*) using a combination of two next-generation sequencing platforms along with a de-

tailed comparative bacterial artificial chromosome (BAC) physical contig map. Heterozygosity of the sequenced source genome allowed discovery of more than 600,000 high-quality single-nucleotide variants. Despite this heterozygosity, the current genome assembly (~1.1 Gb) includes 917 Mb of sequence assigned to specific turkey chromosomes. Annotation identified nearly 16,000 genes, with 15,093 recognized as protein coding and 611 as noncoding RNA genes. Comparative analysis of the turkey, chicken, and zebra finch genomes, and comparing avian to mammalian species, supports the characteristic stability of avian genomes and identifies genes unique to the avian lineage. Clear differences are seen in the number and variety of genes of the avian immune system where expansions and novel genes are less frequent than examples of gene loss. The turkey genome sequence provides resources to further understand the evolution of vertebrate genomes and genetic variation underlying economically important quantitative traits in poultry. This integrated approach may be a model for providing both gene and chromosome level assemblies of other species with agricultural, ecological, and evolutionary interest.

Furthermore, in collaboration with researchers from the USDA, I contributed to the de novo assembly and analysis of the ectoparasitic mite *Varroa destructor*, which is a primary pest of domestic honey bee (*Apis mellifera*). This collaboration extended their previous work to sequence another major pest of honey bees, the microsporidian *Nosema ceranae*. These two organisms are especially significant to agriculture because they are two of the most likely agents of honeybee colony collapse disorder, and assembling their genomes enables unique opportunities for understanding the molecular basis of this serious condition. In the current work, they presented an initial survey of the *V. destructor* genome carried out to advance our understanding of *Varroa* biology and to identify new avenues for mite control. The genome size was estimated by flow cytometry to be 565 Mbp, larger than most sequenced insects but modest relative to some other *Acari*. Genomic DNA pooled from ~1000 mites was sequenced to 4.3× coverage with 454 pyrosequencing. The 2.4 Gbp of sequencing reads were assembled into 184,094 contigs with an N50 of 2262 bp, totaling 294 Mbp of sequence after filtering. Genic sequences that have homology with other eukaryotic genomes were identified on 13,031 of these contigs, totaling 31.3 Mbp. Alignment of protein sequence blocks conserved among *V. destructor* and four other arthropod genomes indicated

a higher level of sequence divergence within this mite lineage relative to the tick *Ixodes scapularis*. A number of microbes potentially associated with *V. destructor* were identified in the sequence survey, including ~300 kbp of sequence deriving from one or more bacterial species of the *Actinomycetales*. The presence of this bacterium was confirmed in individual mites by polymerase chain reaction (PCR) assay but varied significantly by age and sex of mites. Fragments of a novel virus related to the Baculoviridae were also identified in the survey. The rate of SNPs in the pooled mites was estimated to be 6.2×10^{-5} per base pair, a low rate consistent with the historical demography and life history of the species.

This survey has provided general tools for the research community and novel directions for investigating the biology and control of *Varroa* mites. Ongoing development of *Varroa* genomic resources will be a boon for comparative genomics of underrepresented arthropods and will further enhance the honey bee and its associated pathogens as a model system for studying host–pathogen interactions.

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Michael Schatz

COMPUTATIONAL GENOMICS

M.Q. Zhang   M. Akerman   D. Lewis   M. Tang
              M. Kato   W. Liao   K. Wrzeszczynski
              J. Kinney   Y. Ma   J. Wu
              D. LaScala-Gruenewald

In the last year, the majority of my lab moved to the University of Texas at Dallas and I became an adjunct professor at CSHL. D. Lewis moved to the McCombie lab, M. Tang moved to the Lucito lab, K. Wrzeszczynski moved to the Hicks lab, and M. Kato moved to the Krasnitz lab. J. Kinney has become a CSHL Computational Junior Fellow. Miss Diana LaScala-Gruenewald was our URP student from Massachusetts Institute of Technology, and we had visiting Ph.D. student Yu Liu from Tsinghua University working in the Huang lab on a joint project. M. Akerman and J. Wu remain at CSHL working closely with the Krainer lab. W. Liu and Y. Ma also remain at CSHL to work on the STARR project in collaboration with Dr. Ming Li at Memorial Sloan-Kettering Cancer Center.

Comparison of Sequencing-Based Methods to Profile DNA Methylation and Identification of Monoallelic Epigenetic Modifications

Analysis of DNA methylation patterns relies increasingly on sequencing-based profiling methods. The four most frequently used sequencing-based technologies are the bisulfite-based methods MethylC-seq and reduced representation bisulfite sequencing (RRBS), and the enrichment-based techniques methylated DNA immunoprecipitation sequencing (MeDIP-seq) and methylated DNA-binding domain sequencing (MBD-seq). In collaboration with the NIH Roadmap Epigenome Comsortium, we applied all four methods to biological replicates of human embryonic stem cells to assess their genome-wide CpG coverage, resolution, cost, concordance, and the influence of CpG density and genomic context. The methylation levels assessed by the two bisulfite methods were concordant (their difference did not exceed a given threshold) for 82% for CpGs and 99% of the non-CpG cytosines. Using binary methylation calls, the two enrichment methods were 99% concordant and regions assessed by all four methods were 97% concordant. We combined MeDIP-seq with methylation-sensitive restriction enzyme (MRE-seq) sequencing for comprehensive methylome coverage at lower cost. This, along with RNA-seq and ChIP-seq of the embryonic stem (ES) cells, enabled us to detect regions with allele-specific epigenetic states, identifying most known imprinted regions and new loci with monoallelic epigenetic marks and monoallelic expression (Harris et al. 2010). In particular, we show that our new version of software RMAP—which implements function for pair-end and bisulfite next-generation sequencing reads (Smith et al., *Bioinformatics 25:* 2841 [2009])—is more sensitive than *Bowtie*, a popular reads mapping software.

Development of the Human Cancer microRNA Network

In collaboration with Drs. Bandyopadhyay and Mitra at the Indian Statistical Institute, a cancer-miRNA (microRNA) network (Bandyopadhyay et al. 2010) was developed by mining the literature of experimentally verified cancer-miRNA relationships. This network reveals several new and interesting biological insights that were not evident in individual experiments, but become evident when studied in the global perspective. From the network, a number of cancer-miRNA modules have been identified based on a computational approach to mine associations between cancer types and miRNAs. The modules that are generated based on the association are found to have a number of common predicted target onco/tumor suppressor genes. This suggests a combinatorial effect of the module-associated miRNAs on target gene regulation in selective cancer tissues or cell lines. Moreover, neighboring miRNAs (a group of miRNAs located within 50 kb of genomic location) of these modules show similar dysregulation patterns, suggesting a common regulatory pathway. In addition, neighboring miRNAs may also show similar dysregulation patterns (differentially coexpressed) in the cancer tissues. In this

study, we found that 67% of the cancer types have at least two neighboring miRNAs showing down-regulation that is statistically significant ($P < 10^{-7}$, randomization test). A similar result is obtained for the neighboring miRNAs showing up-regulation in a specific cancer type. These results elucidate the fact that neighboring miRNAs might be differentially coexpressed in cancer tissues as those in the normal tissue types. Additionally, the cancer-miRNA network efficiently detects hub miRNAs dysregulated in many cancer types and identify cancer-specific miRNAs. Depending on the expression patterns, it is possible to identify those hubs that have strong oncogenic or tumor suppressor characteristics. This work was based on our new *SFSSClass*, an integrated method for miRNA-based tumor classification (Mitra et al. 2010).

Dissecting the Unique Role of the Retinoblastoma Tumor Suppressor during Cellular Senescence

The retinoblastoma (RB) protein family (RB, p107, and p130) has overlapping and compensatory functions in cell cycle control. However, cancer-associated mutations are almost exclusively found in RB, implying that RB has a nonredundant role in tumor suppression. In collaboration with the Lowe lab at CSHL, we demonstrated that RB preferentially associates with E2F target genes involved in DNA replication and is uniquely required to repress these genes during senescence but not other growth states. Consequently, RB loss leads to inappropriate DNA synthesis following a senescence trigger and, together with disruption of a p21-mediated

cell cycle checkpoint, enables extensive proliferation and rampant genomic instability. Our results (Chicas et al. 2010) identified a nonredundant RB effector function that may contribute to tumor suppression and reveal how loss of RB and p53 cooperate to bypass senescence.

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COLD SPRING HARBOR LABORATORY FELLOWS

In 1986, Cold Spring Harbor Laboratory began a Fellows Program to encourage independent research by outstanding young scientists who, during their graduate studies, displayed exceptional promise of becoming leading scientists of the future. The purpose of this program is to provide an opportunity for these young scientists to work independently at the Laboratory for a period of 3 years on projects of their own choosing. Fellows are provided with a salary, research support, and technical assistance so that they can accomplish their goals free of distraction. The interactions among research groups at the Laboratory and the program of courses and meetings on diverse topics in biology contribute to a research environment that is ideal for innovative science by these Fellows. The CSHL Fellows Program has been tremendously successful and has served as a paradigm for several analogous programs at other institutions, most recently a Fellows Program sponsored by the National Institutes of Health.

The success of the program is apparent from the list of distinguished alumni. Most notably, Carol Greider, recipient of the 2009 Nobel Prize in Physiology or Medicine for her work on telomerase and telomere function, joined the Fellows Program in 1998. After completing her fellowship, Carol was on the CSHL faculty for 9 years, and she is currently the Daniel Nathans Professor and Director of Molecular Biology and Genetics at Johns Hopkins University School of Medicine.

Previous CSHL Fellows Adrian Krainer (1986), Scott Lowe (1995), and Marja Timmermans (1998) are now professors at the Laboratory. Scott is the Deputy Director of the CSHL Cancer Center and a Howard Hughes Medical Institute Investigator, and Marja is the Director of the Fellows Program. Eric Richards (1989) currently is the Vice President of Research and Senior Scientist at the Boyce Thompson Institute for Plant Research at Cornell University; David Barford (1991) is a Fellow of the Royal Society and Professor of Molecular Biology at the Institute of Cancer Research in London; Ueli Grossniklaus (1994) is Professor at the Institute of Plant Biology, University of Zürich, Switzerland; and Térence Strick (2000) left at the end of his fellowship to become a Group Leader at the Institute Jacques Monod in Paris. Both Lee Henry (2000) and Ira Hall (2004) moved to Virginia upon completion of their fellowships. Lee joined a project headed by Thomas Südhof at HHMI's Janelia Farm in Ashburn, and Ira is Assistant Professor in the Department of Biochemistry & Molecular Genetics at the University of Virginia in Charlottesville. Patrick Paddison, who had joined the Fellows Program in 2004, currently is an Assistant Member at the Fred Hutchinson Cancer Research Center in Seattle, Washington.

In 2010, the Laboratory hosted two Fellows: Florin Albeanu did his graduate studies at Harvard University in the laboratories of Venki Murthy and Markus Meister, where he investigated the logic of odor maps in the olfactory bulb of rodents. As a Fellow, Florin has taken advantage of optical imaging, electrophysiology, and optogenetic tools to understand what computations occur at the neural circuit level in the olfactory bulb of behaving mice. His research program has been very successful and he has therefore recently been promoted to Assistant Professor. Chris Vakoc joined us from Gerd Blobel's laboratory at the University of Pennsylvania, where he studied chromatin looping mechanisms in long-range enhancer function. As a Fellow, Chris is studying the role of histone lysine methylation in normal and malignant hematopoiesis.

Understanding Neuronal Circuits in the Mammalian Olfactory Bulb

F. Albeanu, H.G. Chae, P.G. da Silva, M. Davis, A. Dhawale, F. Marbach, H. Patel, S. Rengarajan

In many regions of the brain, neurons form an ordered representation of the outside world. For example, the

"homunculus" of the somatosensory cortex is a point-to-point topographic map of the body surface onto the brain surface. The spatially organized convergence of sensory inputs often leads to similar response properties in target neurons that are in close vicinity. Whether their individual information content is redundant or independent depends on the circuit architecture (the interplay between

common input, lateral signals, and feedback from other brain areas) and the computational goals of the network.

We use optogenetic tools (such as two-photon imaging of genetically encoded neuronal activity reporters or light-gated neuronal activity switches) coupled with electrophysiological measurements (extracellular and intracellular recordings) to study how sensory systems encode information from the environment. We want to understand how these inputs get processed at different junctions or synapses of the underlying neuronal circuits and how these representations change with the state of the system and its circuits (awake vs. anesthetized, naïve vs. conditioned). The broad scope of this effort is observing how perceptions arise.

The olfactory system, particularly the olfactory bulb (OB), in rodents provides us with an ideal substrate to answer these questions, given its well-defined circuitry and multilayered organization. Rodents, being nocturnal animals, depend heavily on olfaction for survival—finding food and mates or avoiding predators. Airborne chemicals or odors are translated into neuronal signals by specific receptors in the nose and sent first to the bulb and then to higher centers in the brain (olfactory cortex). The bulb thus being the relay center provides the opportunity to study both the nature of the inputs it receives and the nature of output it sends to the brain and the computations that allow for this input–output transformation.

In the bulb, sensory neurons expressing the same type of olfactory receptor converge in tight focus, forming ~2000 clusters of synapses called glomeruli. The layout of glomeruli on the bulb is highly reproducible across individuals with a precision of one part in 1000. However, nearby glomeruli are as diverse in their responses to odors as distant ones, lacking an apparent chemotopic arrangement (Soucy et al., *Nat Neurosci 12:* 2 [2009]). From each glomerulus, a few dozen mitral cells (principal output neurons of the OB) carry the output further to the olfactory cortex and several other brain areas. The mitral cells typically have only one primary dendrite that projects to a single glomerulus, but they can sample inputs on their primary and secondary dendrites from functionally diverse glomeruli via several types of interneurons (Fig. 1). Thus, a few dozen mitral cells share input from the same parent glomerulus (sister cells), but they may have different inhibitory surrounds.

Are Outputs from the Same Glomerulus Redundant?

We generated transgenic mice expressing channelrhodopsin-2 in all olfactory sensory neurons under the control of
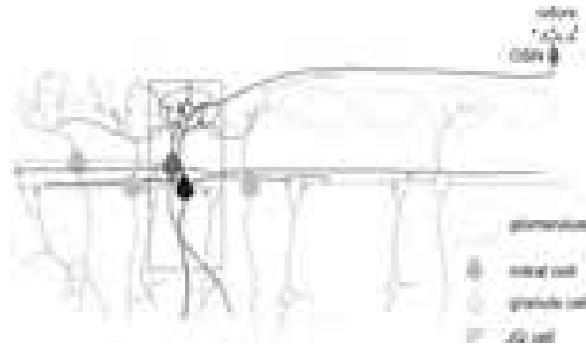
Figure 1. Main olfactory bulb wiring schematics. (Courtesy of F. Markopoulos.)

the olfactory marker protein (OMP) promoter. By selectively stimulating individual glomeruli via DLP-patterned illumination and recording extracellularly from the mitral cell layer using tetrodes, we identified mitral cells that receive common input (Fig. 2). We found that sister mitral cells had highly correlated responses to odors as measured by average spike rates, but their spike timing with respect to respiration was differentially altered. In contrast, non-sister mitral cells correlated poorly on both these measures. Sister mitral cell pairs fired synchronously (at the same phase) within a respiration cycle at rest, and upon odor presentation, they became desynchronized in a stimulus-specific manner. We therefore propose that sister cells carry two different channels of information to the cortex: (1) average activity representing shared glomerular input and (2) phase-specific information that refines odor representations and is substantially independent for sister mitral cells (Dhawala et al., *Nat Neurosci 13:* 1404–1412 [2009]).

What Roles Does the Inhibitory Network Have in Shaping the Bulb Outputs?

Activity in the bulb is a rich mix of excitation and inhibition, via both direct inputs and feedforward and feedback connections by a wide variety of cell types. Juxta-glomerular cells are local interneurons that surround the glomeruli and send neurites within and across glomeruli. In the external plexiform layer, another class of interneurons, the granule cells, mediates cross-talk between mitral cells via reciprocal synapses with lateral mitral cell dendrites (Fig. 2). Both interneuron types are generic denominations for heterogeneous neuronal populations with respect to wiring, gene expression, and physiological properties. Despite decades of study in the slice preparation, little is known about how these interneurons operate in the intact circuit in vivo.
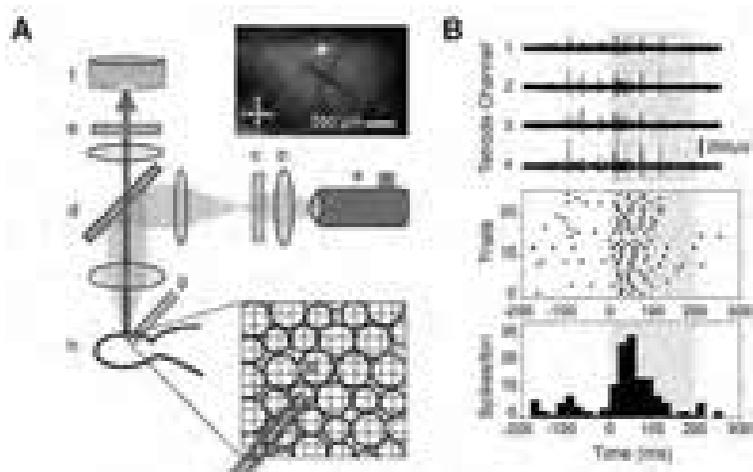
Figure 2. DLP patterned illumination in ORC mice maps the parent glomeruli of mitral cells in vivo. (*A, Left*) A DLP projector coupled to a system of three lenses delivered light patterns to activate individual glomeruli on the dorsal surface of the bulb. A CCD (charge-coupled device) camera was used to visualize the bulb surface and record the position of the light stimulus. (*Top panel, right*) Dorsal surface of the bulb with a tetrode positioned in the mitral cell layer. One square light spot is being projected onto the surface of the bulb. (*Inset*) Cartoon schematic of glomeruli on the bulb, a subglomerular-size light spot and dual tetrodes positioned in the mitral cell layer. (*a*) DLP projector, with color wheel removed from optical path, (*b*) focusing lens, (*c*) blue excitation filter, (*d*) dichroic mirror, (*e*) emission filter, (*f*) CCD camera, (*g*) dual-tetrode, (*h*) olfactory bulb. (*B, Top*) Raw voltage traces corresponding to the four channels of a tetrode showing light-induced changes in firing patterns of the units recorded. (*Center*) Raster plot shows changes in spiking activity of a single isolated unit across 25 light-stimulation trials, each 200 msec long; dots mark spike occurrence times. (*Bottom*) Peristimulus time histogram (PSTH) with 25-msec time bins, summing spikes over the trials shown in the center panel.

First, we aimed at understanding whether bulb interneurons modulate the spiking time of mitral cells, i.e., the changes in phase tuning described above. We have started by expressing light-gated molecular switches of neuronal activity (such as channelrhodopsin 2 and halorhodopsin) in genetically defined subsets of bulb interneurons. We are using a Cre/Lox strategy and taking advantage of several interneuron-specific Cre lines generated by Josh Huang's group at CSHL. In particular, we are targeting local dopaminergic interneurons in the glomerular layer (tyrosine-hydroxylase-positive) that mediate interglomerular cross-talk, as well as deeper interneurons situated in the external plexiform layer (parvalbumin and or VIP-positive) that synapse with the lateral dendrites of mitral cells.

Optical Dissection of Olfactory Microcircuits: What Computations Are Performed on the Inputs by the Bulb Circuitry?

We set out to sample activity of large and diverse neuronal populations from the two layers of the bulb in response to numerous odorants. Recent developments in

multiphoton microscopy and new generations of genetically encoded neuronal activity reporters allow monitoring both glomeruli (inputs) and mitral cell (outputs) responses simultaneously within the same animal and in response to the same set of odorants. Toward this end, we built two custom multiphoton microscopes that allow for fast *x-y-z* scanning and two large-scale odor delivery machines (165 odorants). Preliminary experiments indicate that activity patterns at the level of mitral cell bodies are not simply scaled down versions of glomerular representations (Fig. 3). Therefore, we have started to systematically investigate the contributions of lateral signals and feedback from other areas to bulb input-output transform via optogenetic strategies.

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

We are using light activation of glomeruli in awake head-restrained OMP-chr2 mice to ask how different spatiotemporal input patterns get processed at the level of the mitral cells and influence behavior. Using light activation
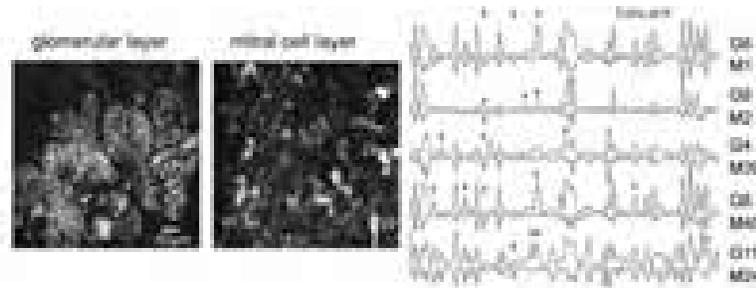
Figure 3. Odor responses of glomerular and mitral cell body layers via multi-photon imaging of GCAMP2 signals. (*Left*) Glomeruli, (*center*) mitral cell bodies, (*right*) best functional matches between glomerular and mitral cell bodies odor responses to a panel of 125 stimuli. (G) Glomerulus; (M) mitral cells from Kv3.1 GCAMP2 mice (Fletcher et al., *J Neurophysiol 102,* 817 [2009]).

of the glomeruli bypasses the inherent uncertainty of olfactory stimuli delivery, as odor plumes diffuse slowly and in complex patterns. Our goal is to generate olfactory percepts by strictly controlled patterned light stimulation and dissect the neurocorrelates of those patterns that dictate olfactory behavior at the level of olfactory bulb circuitry. Using the strategy described above, we are stimulating select sets of glomeruli with a combination of blue and orange light via the DMD-based patterned illumination/ multiphoton imaging rig. The mice are trained to identify this particular pattern, called "A" in a two alternative forced-choice (2AFC) tasks as being unique from any other stimulus pattern "NOT A." For instance, when studying interglomerular timing as a feature, "NOT A" may contain variations of "A" with altered intensities, added or dropped glomeruli, "A" in presence of foster odors, etc. Posttraining, probe trials are conducted in which only the feature of interest (timing in the above described case) is varied. The behavior is monitored for errors in classification and differences in response latencies. As a second-level perturbation, different subsets of interneurons are inhibited/activated over distinct time windows while stimulating with pattern "A," and any changes in behavior are determined. The behavioral effects imposed by these two kinds of perturbations will then be compared to elucidate which subsets of interneurons mediate the processing of that particular feature of the odor code.

Other Collaborative Projects with CSHL Groups

We are colloborating with fellow scientists on the following projects: optical monitoring of cholinergic modulation of cortical circuits (with A. Kepecs); bridging the gap between the functional responses of glomeruli to odors and odor receptor sequences (with A. Koulakov);

monitoring synaptic plasticity of bulb circuits and AMPA receptor trafficking in real time, during learning (with B. Li); optical monitoring of neuronal activity in genetically and anatomically defined cortical circuits in animal models of cognition (with A. Zador); using optical tools to study molecular mechanisms controlling branching of maize inflorescence (with D. Jackson).

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Analysis of Complex Phenotypes via Molecular Networks and New-Generation Sequencing

I. Iossifov

I am studying the genetics of common diseases in humans using two main tools: new-generation sequencing and molecular networks representing functional relationships among genetic loci. In combination, these tools enable large-scale studies necessary for furthering our understanding of the complex etiology of disorders such as autism, bipolar disorder, and cancer. I focus both on the development of new methods and tools—for sequence analysis, for building, and for using molecular networks—and on their application for specific biomedical problems.

ONGOING PROJECTS

Whole-Exome Sequencing of the SSC

The Simons simplex collection (SSC) is expected to grow to 4000 families, with one child diagnosed with

autism spectrum disorder and with at least one unaffected child. Such a collection is ideally suited for identification of de novo mutations with a strong effect on the disorder. Recent improvements of the techniques for enrichment of coding genomic sequences and of the new-generation sequencing technology made feasible whole-exome sequencing for a large number of samples. The generated data are of high quality and can be used to identify de novo single-nucleotide, short-indel, and copy-number mutations. This project was carried out in collaboration with Michael Wigler and Richard McCombie at CSHL.

Analysis of the Pathways Affected by De Novo Copy-Number Mutations Identified in 2000 Families from the SSC Collection

Recent evidence suggests that rare variants, including copy-number variants (CNVs), have a significant role in the etiology of autism. Although many such variants have been identified, the specific molecular networks associated with this complex disorder remain largely unknown. In this study, we develop a method for network-based analysis of genetic associations (NETBAG). We use NETBAG to identify a large biological network of genes affected by rare de novo CNVs in autism. The genes forming the network are primarily related to synapse development, axon targeting, and neuron motility. This project is being carried out in collaboration with Michael Wigler (CSHL) and Dennis Vitkup (Columbia University).

Analysis of the Overall Sharing of Parental Genome by Concordant and Discordant Autistic Siblings

The goal is to test the hypothesis that a substantial proportion of inherited autism is caused by a dominant mutation inherited from the mother, with different mothers having different affected loci. So far, we see a suggestive increase of sharing of the mothers' genomes in a cohort of ~300 multiplex families from the AGRE collection with microsatellite markers. The next step is to see if the signal is preserved and statistically significant in a larger set of 700 families genotyped with a 10K single-nucleotide polymorphism (SNP) array. We are carrying out this project in collaboration with Michael Wigler and Kenny Ye at CSHL.

Sequencing of Treatment-Related tAML 5q and 7q Chromosomes

A large proportion of treatment-related acute myeloid leukemia (tAML) cases present with hemizygous 7q or 5q chromosomes. In collaboration with Mona Spector and Scott Lowe at CSHL, we are sequencing 50 samples with the hope of identifying a gene within the 5q and 7q regions with recurrent variants affecting protein sequence.

Epigenetics and Cancer
C.R. Vakoc

Chromatin is the fundamental packaging material of eukaryotic genomes, composed of DNA in its native complex with protein. The basic organizational unit of chromatin is the nucleosome, composed of an octamer of core histones wrapped by 147 bp of DNA. Chromatin is charged with the critically important task of both protecting the genetic code and animating its many functions. Replication, recombination, and transcription of DNA in the cell all occur in a chromatin context, and hence, elaborate control mechanisms have evolved to govern chromatin structure in these diverse scenarios. The central interest of our laboratory is to understand the enzymatic mechanisms that control chromatin and how such pathways establish heritable chromatin states and participate in the pathophysiology of cancer.

A major focus of our work is a class of molecules referred to as human Trithorax proteins, also known as the mixed-lineage leukemia (MLL) family. MLL proteins are large, modular chromatin regulators that use catalytic (methyltransferase activity for histone H3 lysine 4) and noncatalytic (chromatin-binding and scaffold functions) mechanisms to maintain the "ON" state of gene expression. One fascinating feature of MLL and its orthologs in other species is their capacity to maintain heritable states of gene activity, as exemplified by MLL in *Drosophila* maintaining heritable programs of *Hox* gene expression, a phenomenon known as cellular memory. Although most of the classic epigenetic inheritance phenomena have been linked with the "OFF" state of expression, the epigenetic maintenance of the "ON" state by MLL stands alone as one of the only known mechanisms in nature that stably preserves active chromatin in a manner heritable through somatic cell divisions. One key interest in our laboratory is to define the molecular mechanism of epigenetic inheritance by MLL proteins.

In addition to having essential functions in maintaining active chromatin during normal ontogeny and dif-

ferentiation, the MLL protein is also a proto-oncogene in acute leukemia. MLL is frequently mutated via chromosomal translocation to generate oncogenic fusion proteins. MLL fusion proteins induce a hyperactive chromatin state as compared to wild-type MLL, which leads to up-regulation of MLL's normal target genes to block hematopoietic cell differentiation and induce leukemia. Thus, MLL leukemias represent a clear situation where chromatin dysfunction directly causes a human cancer. A major thrust of our research is to use MLL leukemia as a model system to study how epigenetic pathways participate in cancer pathogenesis. Our primary approach is to evaluate whether therapeutic targeting of epigenetic pathways can eradicate leukemia cells in vivo. Because MLL leukemia represents a poor-prognosis subtype of this disease, we hope to identify novel therapeutic targets in this cancer by mining epigenetic pathways.

RNAi Screening to Expose Epigenetic Vulnerabilities in AML

E. Wang, J. Shi

The central hypothesis our work on acute myeloid leukemia (AML) is that suppression of epigenetic pathways can be an effective means to achieve selective eradication of leukemia cells in vivo. However, the challenge is in how one identifies candidate epigenetic targets as novel cancer therapies. To this end, we have begun RNA interference (RNAi) screening as a means of revealing and distinguishing the epigenetic requirements between normal and malignant cells. First, we generated a custom short hairpin RNA (shRNA) library targeting all known chromatin regulators, which includes ~1100 shRNAs targeting 250 genes. Next, we introduced this library in a 1-
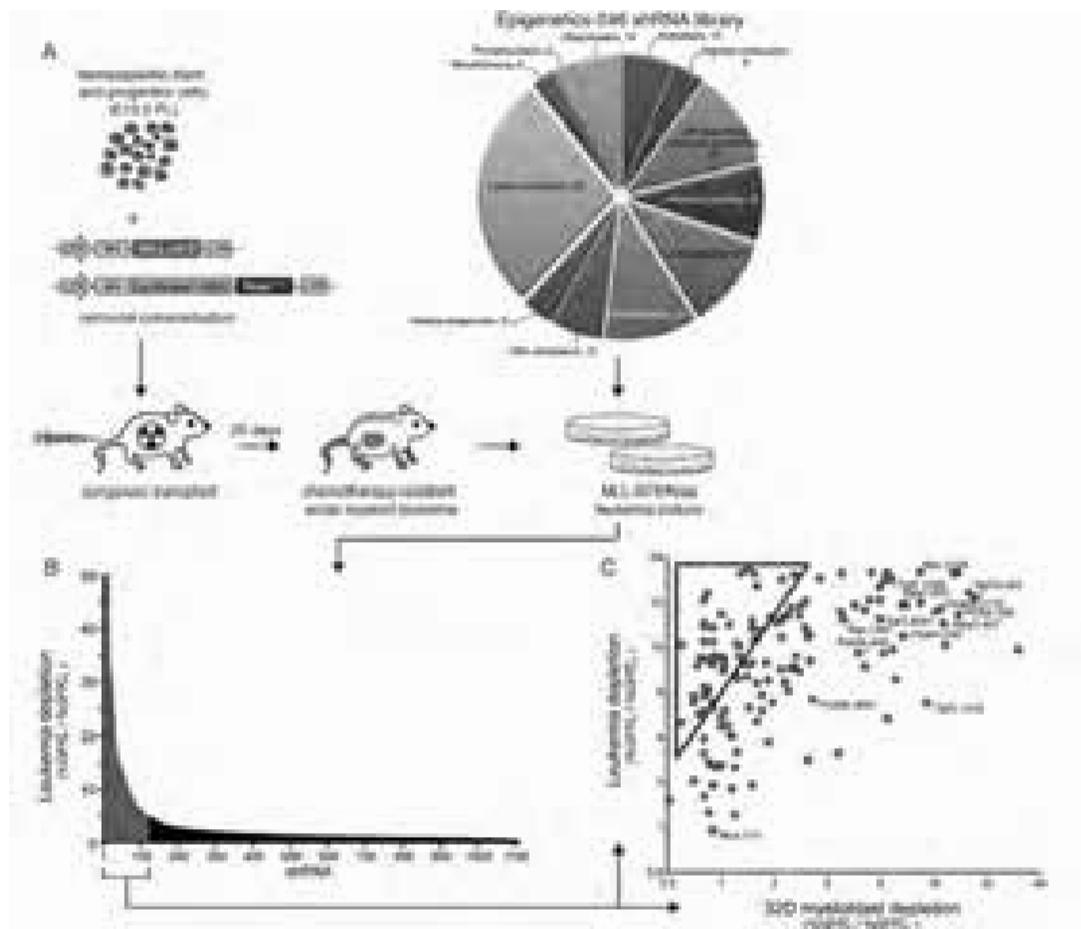
Figure 1. RNAi screen for unique epigenetic requirements needed for MLL-leukemia growth. (*A*) Experimental strategy. A mosaic mouse model of MLL–AF9/Nras^{G12D} was transduced with the Epigenetics-246 shRNA library. (*B*) Primary screen identified 129 shRNAs that inhibit proliferation (in gray) (*C*) Comparison of shRNA effects in leukemia and 32D myeloid contexts. The triangle demarcates shRNAs that tested positive in the screen by preferentially inhibiting leukemia proliferation. Positive control shRNAs are labeled.

by-1 or pooled fashion into a mouse model of MLL–AF9/ Nras^{G12D} leukemia. We then scored the impact of each shRNA on AML growth in vitro and compared the effects on a number of nontransformed hematopoietic cell lines, representing normal cells. Through this approach, we were able to identify several candidate shRNA pathways, which we want to validate in vivo. Using a similar "negative-selection" strategy, we introduced shRNAs targeting various epigenetic pathways into normal or leukemic cells and transplanted into lethally or sublethally irradiated recipients. The impact of each shRNA was scored using a two-color fluorescence-activated cell sorting (FACS)-based assay. This analysis reliably distinguished the in vivo growth requirement for normal and malignant cells and identified several candidate epigenetic pathways.

Among the top hits in the RNAi screen were the *Eed* and *Suz12* genes, which both encode subunits of the PRC2 (Polycomb repression complex). PRC2 is a histone methyltransferase that selectively modifies histone H3 at lysine 27. This chromatin modification has a clear role in gene repression, which it serves as a docking site for other repressive complexes. Identifying two subunits in the screen provides strong evidence that this complex was involved in AML progression. Following knockdown of PRC2 in MLL-AF9/Nras^{G12D} leukemia cells, we observed robust differentiation of leukemic blasts into cells with an obvious macrophage morphology, indicating that PRC2 is required to prevent differentiation of leukemic cells. This phenotype bears striking resemblance to suppressing MLL-AF9, the driver oncogene in this disease. To identify the core downstream targets of PRC2 in AML, we performed expression microarrays comparing evaluating global changes in gene expression signatures, followed by large-scale chromatin immunoprecipitation experiments to distinguish direct from indirect targets. Using a combination of overexpression and loss-of-function approaches, we were able to pinpoint the Ink4a/Arf tumor suppressor locus as the key downstream target of PRC2 in AML. In addition, we were also able to demonstrate that the oncogenic drivers of AML in this model, MLL-AF9 and Nras^{G12D}, both provide a signal that stimulates Ink4a/Arf expression, which threatens to inhibit growth of AML cells in vitro. In our model, we speculate that PRC2 provides a buffering system in AML for diminishing oncogenic stress. By suppressing PRC2 with RNAi, we eliminate this buffer and unravel the transformed cellular state through induction of Ink4a/ Arf expression. We speculate that PRC2 will represent a promising new therapeutic target in AML.

Another hit identified in the RNAi screen is the BET-bromo domain protein Brd4. Notably, among the 250 candidate genes in the screen, Brd4 inhibition displayed the most profound elimination of leukemia cells in vitro, making it the top hit. Like PRC2, suppression of Brd4 leads to similar induction of myeloid differentiation with associated loss of leukemia stem cell populations. Quite fortunately, our studies have converged with those of the James Bradner laboratory at Harvard, which has recently developed small-molecule inhibitors of the Brd4 bromo-domains. In collaboration with Bradner and Scott Lowe's laboratory at CSHL, we have begun investigating the antileukemic activity of Brd4 inhibitors in vitro and in vivo. This example represents the overall effort in the lab of identifying epigenetic sensitivities in cancer that might be exploited using small-molecule inhibitors, which could be developed in the future as drugs in AML.

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Massachusetts Institute of Technology

WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

Yet another year has flown by here at the Watson School of Biological Sciences (WSBS). In 2010, we were extremely fortunate to recruit Dr. Keisha John back to the School as our new Associate. Keisha was in the School's Entering Class of 2004 and did her doctoral research with Dr. Linda Van Aelst. She graduated in April 2009 and was a postdoctoral fellow with Dr. Mary Beth Hatten at The Rockefeller University. Keisha's professional interests moved in the direction of education administration and we are thrilled to have her as the newest member of "Team Watson."

Team Watson: (*Left to right*) Alyson Kass-Eisler, Dawn Meehan, Pologruto, Keisha John, Leemor Joshua-Tor, and Kim Geer

Faculty Changes

Five new faculty members joined the Laboratory in 2010: Florin Albeanu, Anne Churchland, Christopher Hammell, Michael Schatz, and Hongwu Zheng.

Dinu Florin Albeanu, an Assistant Professor, first came to CSHL in 2008 as a Cold Spring Harbor Fellow. He is a neuroscientist who uses electrophysiology and imaging to study how sensory input, such as olfaction, encodes information from the environment and ultimately leads to perceptions.

Assistant Professor Anne Churchland was a postdoctoral fellow at the University of Washington before coming to CSHL. Her research focuses on the circuitry connecting sensory processing and motor control. She uses both humans and rodents to study how sensory input leads to decision-making.

Assistant Professor Christopher Hammell was a postdoctoral fellow at the University of Massachusetts Medical School before coming to the CSHL. His research focuses on the regulation of the microRNA machinery, which has an important role in the regulation of gene expression. In particular, he is using the model organism, *Caenorhabditis elegans*, to understand the role of microRNAs in development.

Following a career as a software engineer, Michael Schatz, received his Ph.D. from the Center for Bioinformatics and Computational Biology at the University of Maryland before joining CSHL as an Assistant Professor. His research combines computational biology with advances from high-performance computing to address problems in the field of genomics.

Before coming to CSHL as an Assistant Professor, cancer biologist Hongwu Zheng was an Instructor of Medicine at the Dana-Farber Cancer Institute. He uses multiple approaches in his research to study the causal relationship between aberrant differentiation and gliomagenesis. He applies this knowledge to target differentiation control pathways as a novel approach for treating malignant gliomas.

We are very excited to have each of these exciting newcomers work with our students. They have already become members of student thesis committees, and instructors and guest lecturers in our fall courses. We look forward to their participation in additional WSBS activities in the future.

Just one faculty member departed the Laboratory in 2010: Michael Zhang. Michael served as an instructor in the first entering class' Fundamentals of Bioinformatics Specialized Disciplines course. He also served on student thesis committees, as a rotation advisor, and on the admissions committee. We are grateful for his contributions and wish him well in his new endeavor.

Oliver Fregoso, Colin Malone, Yaniv Erlich, Oliver Tam, Amy Leung, Katherine McJunkin, Galen Collins, Hiroshi Makino, David Simpson, Shraddha Pai (*not pictured*)

The Seventh WSBS Graduation

On April 25, we celebrated the Watson School's seventh and largest graduation ceremony to date. The 10 (!) graduating students of 2010 are Galen Collins, Oliver Fregoso, Shraddha Pai, and David Simpson from the entering class of 2004; Amy Leung, Hiroshi Makino, Katie McJunkin, and Oliver Tam from the entering class of 2005; and Yaniv Erlich and Colin Malone from the entering class of 2006. All were awarded the Ph.D. degree. Honorary degrees were bestowed upon Dr. Carla Shatz from Stanford University and Nobel laureate Dr. Tom Cech from the University of Colorado, Boulder, who also gave the commencement address.

As with each graduation, we extended a special welcome to the family members and friends of our students who attend the ceremony. Among these special guests were family members who traveled from Australia, Israel, and Japan to take part in this special event.

Teaching Award

At this year's graduation, Josh Dubnau was presented with the fifth annual Winship Herr Faculty

Teaching Award named in honor of the School's founding dean. Josh was the lead instructor of our fall Specialized Disciplines course in Genetics and Genomics. He was chosen by the students for this award, based on his enthusiasm, excellence, and creativity in teaching. This is the second time Josh has won this award—he was also recognized in 2006. The winner of this award is nominated and voted on by the students. Here are some of the comments the students had to say about Josh:

He mixed historical context, current techniques, and a clear explanation of concepts together with a touch of humor that enabled me and the other students to grasp the information on first pass.

He clearly worked hard on putting together the lectures and homeworks and was very helpful and accessible when additional clarification was needed.

Josh Dubnau

2010 WSBS DOCTORAL RECIPIENTS

Student	Thesis advisor	Academic mentor	Current position
Galen Collins	William Tansey	Marja Timmermans	Postdoctoral fellow with Dr. Fred Goldberg at Harvard Medical School
Yaniv Erlich	Gregory Hannon	John R. Inglis	Whitehead Fellow, Whitehead Institute, Massachusetts (Independent Position)
Oliver Fregoso	Adrian R. Krainer	Nicholas Tonks	Postdoctoral fellow in Dr. Michael Emerman's laboratory at Fred Hutchinson Cancer Research Center, Washington (to start early 2011)
Amy Leung	William Tansey	David L. Spector	Postdoctoral fellow with Dr. Rama Natarajan, City of Hope, California
Hiroshi Makino	Roberto Malinow	Hollis Cline	Postdoctoral fellow with Dr. Yang Dan at the University of California, Berkeley
Colin Malone	Gregory Hannon	David J. Stewart	Postdoctoral fellow with Dr. Ruth Lehmann at The Skirball Institute of Biomolecular Medicine, New York University School of Medicine
Katherine McJunkin	Scott Lowe	Terri Grodzicker	Postdoctoral fellow with Dr. Victor Ambross at University of Massachusetts Medical Center
Shraddha Pai	Carlos D. Brody	Anthony Zador	Postdoctoral fellow with Dr. Arturas Petronis at the Centre for Addiction and Mental Health, University of Toronto, Canada
David Simpson	William Tansey	Scott Lowe	Postdoctoral fellow with Dr. Alejandro Sweet-Cordero at Stanford University (to begin early 2011)
Oliver Tam	Gregory Hannon	David Jackson	Postdoctoral fellow with Dr. Janet Rossant at The Hospital for Sick Children, University of Toronto, Canada

2010 THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2004

Galen Collins, March 15, 2010

Activator turnover and proteolysis in transcriptional activation.

Thesis Examining Committee
Chair: **David L. Spector**
Research mentor: **William Tansey**
Academic mentor: **Marja Timmermans**
Committee member: **Adrian R. Krainer**
Committee member: **Michael Hampsey,** *Robert Wood Johnson Medical School*
External examiner: **Bruce Futcher,** *Stony Brook University*

Oliver Fregoso, February 25, 2010

Elucidating the functions of splicing regulatory proteins through the use of high-throughput proteomics.

Thesis Examining Committee
Chair: **Scott Powers**
Research mentor: **Adrian R. Krainer**
Academic mentor: **Nicholas Tonks**
Committee member: **Michael P. Myers**
Committee member: **Gregory Hannon**
Committee member: **Brian Chait,** *The Rockefeller University*
External examiner: **Alan M. Zahler,** *University of California, Santa Cruz*

Shraddha Pai, January 14, 2010

Neuroanatomical localization of timing-specific brain loci in the rat.

Thesis Examining Committee
Chair: **Josh Dubnau**
Research mentor: **Carlos D. Brody**
Academic mentor: **Anthony Zador**
Committee member: **Partha Mitra**
Committee member: **Lizbeth Romanski,** *The Rockefeller University*
External examiner: **Mark Laubach,** *Yale University*

David Simpson, March 16, 2010

Regulation of myc-*induced apoptosis in the mammary epithelia.*

Thesis Examining Committee
Chair: **Gregory Hannon**
Research mentor: **William Tansey**
Academic mentor: **Scott Lowe**
Committee member: **Senthil K. Muthuswamy**
Committee member: **George Prendergast,** *Lankenau Institute for Medical Research*
External examiner: **Steven McMahon,** *Kimmel Cancer Center*

2010 THESIS DISSERTATION DEFENSES (*continued*)

ENTERING CLASS OF 2005

Amy Leung, March 5, 2010

Regulation of chromatin by histone H2B ubiquitylation.

Thesis Examining Committee
Chair:	**David Jackson**
Research mentor:	**William Tansey**
Academic mentor:	**David L. Spector**
Committee member:	**Robert Martienssen**
Committee member:	**Rolf Sternglanz,** *Stony Brook University*
External examiner:	**Mary Ann Osley,** *University of New Mexico*

Hiroshi Makino, March 8, 2010

AMPA receptor dynamics and synaptic plasticity at excitatory synapses.

Thesis Examining Committee
Chair:	**Anthony Zador**
Research mentor:	**Roberto Malinow**
Academic mentor:	**Bo Li**
Committee member:	**Pavel Osten**
Committee member:	**Linda Van Aelst**
External examiner:	**Edward Ziff,** *New York University*

Katherine McJunkin, February 25, 2010

Inducible RNAi targeting essential genes.

Thesis Examining Committee
Chair:	**Gregory Hannon**
Research mentor:	**Scott Lowe**
Academic mentor:	**Terri Grodzicker**
Committee member:	**Raffaella Sordella**
External examiner:	**Vivek Mittal,** *Memorial Sloan-Kettering Cancer Center*
External examiner:	**Andrea Ventura,** *Cornell University Medical College*

Oliver Tam, February 11, 2010

Characterization of small RNA populations and DNA methylation in mammalian development.

Thesis Examining Committee
Chair:	**Marja Timmermans**
Research mentor:	**Gregory Hannon**
Academic mentor:	**David Jackson**
Committee member:	**Zachary Lippman**
Committee member:	**Richard Schultz,** *Stony Brook University*
External examiner:	**Robert A. Taft,** *The Jackson Laboratory*

ENTERING CLASS OF 2006

Yaniv Erlich, March 19, 2010

Compressed sequencing.

Thesis Examining Committee
Chair:	**Adrian R. Krainer**
Research mentor:	**Gregory Hannon**
Academic mentor:	**John R. Inglis**
Committee member:	**Partha P. Mitra**
Committee member:	**Michael Q. Zhang**
External examiner:	**David Donoho,** *Stanford University*

Colin Malone, February 8, 2010

Evolution, inheritance, and specialization of transposon control pathways in Drosophila.

Thesis Examining Committee
Chair:	**David L. Spector**
Research mentor:	**Gregory Hannon**
Academic mentor:	**David J. Stewart**
Committee member:	**Robert Martienssen**
Committee member:	**Ruth Lehmann,** *New York University*
External examiner:	**Craig Mellow,** *University of Massachusetts*

ENTERING CLASS OF 2010

Arkarup Bandyopadhyay, Tata Institute of Fundamental Research, University of Delhi, India
Academic mentor: Zachary Lippman

Colleen Carlston, Harvard University
Academic mentor: Alexander Gann

Matthew Koh, University of California, Berkeley
Academic mentor: Bo Li

Lisa Krug, Skidmore College
Academic mentor: Stephen Shea

Mark Orcholski, Buck Institute, Dominican University
Academic mentor: Nicholas Tonks

John Sheppard, Northwestern University
Academic mentor: Josh Dubnau

Jack Walleshauser, University of North Carolina, Greensboro
Academic mentor: Christopher Hammell

2010 Entering class: (*Left to right*) Lisa Krug, Arkarup Bandyopadhyay, Colleen Carlston, John Sheppard, Jack Walleshauser, Mark Orcholski, and Matthew Koh.

Admissions 2010

The School received 170 applications for the 2010/2011 academic year and is deeply indebted to its Admissions Committee, which reviewed, interviewed, and selected candidates for our doctoral program. The Admissions Committee for the 2010 entering class comprised Gregory Hannon (chair), Mickey Atwal, Leemor Joshua-Tor, Adam Kepecs, Adrian R. Krainer, Zachary Lippman, W. Richard McCombie, Nicholas Tonks, Lloyd Trotman, and Linda Van Aelst—a truly remarkable team!

Entering Class of 2010

On August 30, 2010, the Watson School opened its doors for the 12th time to welcome yet another new class. This year, seven students joined the School: Arkarup Bandyopadhyay, Colleen Carlston, Matthew Koh, Lisa Krug, Mark Orcholski, John Sheppard, and Jack Walleshauser. Colleen was a participant in the summer Undergraduate Research Program in 2008 under the mentorship of Josh Dubnau.

The 2010 class enjoys a sail on Oyster Bay.

DOCTORAL THESIS RESEARCH

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2005			
Patrick M. Finigan *Beckman Graduate Student*	Senthil K. Muthuswamy	Robert Martienssen	Epigenetic mechanisms involved in centromere function.
Frederick D. Rollins *Cashin Fellow*	Jan A. Witkowski	Gregory Hannon	An RNAi screen for modifiers of cellular response to the targeted therapeutic Erlotinib.
ENTERING CLASS OF 2006			
Eyal Gruntman *Elisabeth Sloan Livingston Fellow*	Josh Dubnau	Glenn Turner	Olfactory perception in *Drosophila*.
Amy Rappaport *Barbara McClintock Fellow* Thesis defense: March 2011	William Tansey	Scott Lowe	Identification and characterization of tumor suppressor genes in acute myeloid leukemia.
Claudio Scuoppo *Engelhorn Scholar* Thesis defense: March 2011	Scott Lowe	Gregory Hannon	Identification of novel oncosuppressors through an in vivo RNAi screen in the Eμ-*myc* model.
ENTERING CLASS OF 2007			
Megan Bodnar *Starr Centennial Scholar*	Nicholas Tonks	David L. Spector	The nuclear choreography of chromatin dynamics, gene expression, and gene repression in embryonic stem cells.
Ralph Burgess *Starr Centennial Scholar*	Bruce Stillman	Gregory Hannon	Recombination hot spots: Characterizing fine-scale variation in the frequency of meiotic recombination across the mammalian genome.
Joseph Calarco *David H. Koch Fellow*	David Jackson	Leemor Joshua-Tor	Transposable element regulation in the flowering plant.
Saya Ebbesen *NIH Predoctoral Trainee*	David J. Stewart	Scott Lowe	In vivo identification and characterization of novel tumor suppressors relevant to human breast cancer.
Paloma Guzzardo *Leslie C. Quick, Jr. Fellow* *NIH Predoctoral Trainee*	Adrian R. Krainer	Gregory Hannon	Dissecting the piRNA pathway in the *Drosophila* ovarian somatic sheet.
Kyle Honegger *Crick-Clay Fellow* *NIH Predoctoral Trainee*	John R. Inglis	Glenn Turner	Neuronal and circuit mechanisms creating sparse odor representations in the mushroom body of *Drosophila*.
Marek Kudla *George A. and Marjorie H. Anderson Fellow*	David Jackson	Gregory Hannon	CLIP method as a way of direct readout of microRNA target sites.
Hassana Oyibo *Farish-Gerry Fellow*	Hiro Furukawa	Anthony Zador	Reconstruction of connectivity of neurons by reading of oligonucleotide labels (ROC-N-ROL).
Michael Pautler *William R. Miller Fellow*	Robert Lucito	David Jackson	The RAMOSA pathway and inflorescence branching in maize.
Maria Pineda *CSHL Women's Partnership for Science Student*	Adrian R. Krainer	Raffaella Sordella	Mechanism of "addiction" to receptor tyrosine kinases in non-small-cell lung carcinoma.
Yevgeniy Plavskin *Alfred Hershey Fellow* *NIH Predoctoral Trainee*	Jan A. Witkowski	Marja Timmermans	The evolution of the miR390-dependent tasiRNA pathway and its function in the bryophyte development.
Joshua Sanders *Edward and Martha Gerry Fellow*	Bruce Stillman	Adam Kepecs	*Trans*-regional coordination of activity in the mouse brain.
Zhenxun Wang *A*STAR Fellow*	Terri Grodzicker	Adrian R. Krainer	Alternative splicing of pyruvate kinase M in tumorigenesis.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Petr Znamenskiy *David and Fanny Luke Fellow*	Terri Grodzicker	Anthony Zador	Pathways for attention and action in the auditory system.

ENTERING CLASS OF 2008

Student	Academic mentor	Research mentor	Thesis research
Philippe Batut *Florence Gould Fellow*	Alexander Gann	Thomas Gingeras	Transposable and evolution of transcriptional regulation in the *Drosophila* clade.
Mitchell Bekritsky *Beckman Graduate Student*	W. Richard McCombie	Michael Wigler	High-throughput microsatellite genotyping.
Dario Bressan *Lindsay Fellow*	Z. Josh Huang	Gregory Hannon	A genomic approach toward the elucidation of connectivity patterns at cellular resolution in complex neural networks.
Carrie Clendaniel *Beckman Graduate Student*	David Jackson	Marja Timmermans	Role of HIRA in the regulation of developmentally important target loci.
Melanie Eckersley-Maslin *Genentech Foundation Fellow*	Gregory Hannon	David L. Spector	Making the choice: Mechanistic insights into random autosomal monoallelic expression in mammalian cells.
Sang Geol Koh *George A. and Marjorie H. Anderson Fellow*	Glenn Turner	Anthony Zador	Attention-dependent information routing in the mouse auditory cortex.
Katie Liberatore *NSF Graduate Research Fellow*	Adrian R. Krainer	Zachary Lippman	Inflorescence development and heterosis.
Ozlem Mert *George A. and Marjorie H. Anderson Fellow*	John R. Inglis	Scott Lowe	Characterization of the role of E2F7 in oncogene-induced senescence and tumorigenesis.
Elizabeth Nakasone *DoD BCRP Predoctoral Fellow*	Alea A. Mills	Mikala Egeblad	Understanding the effects of cytotoxic chemotherapeutics on the innate immune response.
Zinaida Perova *Charles A. Dana Fellow*	Linda Van Aelst	Bo Li	Role of medial prefrontal cortex in behavioral depression.
Felix Schlesinger *Crick-Clay Fellow*	Gregory Hannon	Thomas Gingeras	Classification of novel transcription start sites.
Nilgun Tasdemir *Lindsay Fellow*	Josh Dubnau	Scott Lowe	Investigating the molecular overlaps between epigenetic reprogramming and transformation.
Elvin Wagenblast *Boehringer Ingelheim Fonds Scholar*	Jan A. Witkowski	Gregory Hannon	Role of stem/progenitor cells in mammary gland and breast tumors.
Susann Weissmueller *Annette Kade Fellow*	Raffaella Sordella	Scott Lowe	In vivo identification and characterization of tumor suppressor genes in hepatocellular carcinoma.

ENTERING CLASS OF 2009

Student	Academic mentor	Research mentor	Thesis research
Stephane Castel *Cashin Fellow* Proposal defense: February 2011	Lloyd Trotman	Robert Martienssen	RNAi-mediated heterochromatin in *S. pombe*.
Kristen Delevich *NIH Predoctoral Trainee* Proposal defense: January 2011	Stephen Shea	Bo Li	Elucidating the role of Disrupted-in-Schizophrenia-1 in development of prefrontal cortical circuits.
Silvia Fenoglio *Elisabeth Sloan Livingston Fellow* Proposal defense: December 2010	Linda Van Aelst	Gregory Hannon	RNAi screening to identify putative therapeutic targets for the treatment of pancreatic cancer.
Wee Siong Goh *A*STAR Fellow* Proposal defense: January 2011	Hiro Furukawa	Gregory Hannon	Determining piRNA primary biogenesis, and MIWI and late piRNA function in mice using *C. elegans* as a model system for genetic screening.

	DOCTORAL THESIS RESEARCH (*continued*)		
Student	Academic mentor	Research mentor	Thesis research
Ian Peikon *George A. and Marjorie H. Anderson Fellow* Proposal defense: February 2011	Mickey Atwal	Anthony Zador	Reverse engineering the brain.
Kaja Wasik *George A. and Marjorie H. Anderson Fellow* Proposal defense: February 2011	Jan A. Witkowski	Gregory Hannon	A screen for novel components of the piRNA pathway in *Drosophila melanogaster*.
Cinthya Zepeda Mendoza *Gonzalo Rio Arronte Fellow* Proposal defense: January 2011	Thomas Gingeras	David L. Spector	Analysis of higher-order chromatin organization at the mouse syntenic region of human 1p36 upon genomic copy-number changes.

Academic Mentoring

The Watson School takes great pride in the level of mentoring that it offers its students. One of the very special aspects in this regard is our two-tiered mentoring approach whereby each student receives an academic mentor as well as a research mentor. Entering students select by mutual agreement a member of the research or nonresearch faculty to serve as an academic mentor—a watchful guardian to look over and encourage students through the sometimes-trying process of their doctoral education. This program continues to receive much support from the faculty who volunteer to be academic mentors, and it has rightfully become a vital ingredient in our success. The following are this year's new academic mentors for the entering class of 2010:

STUDENT	MENTOR	STUDENT	MENTOR
Arkarup Bandyopadhyay	Zachary Lippman	Mark Orcholski	Nicholas Tonks
Colleen Carlston	Alex Gann	John Sheppard	Josh Dubnau
Matthew Koh	Bo Li	Jack Walleshauser	Christopher Hammell
Lisa Krug	Stephen Shea		

The Fall Term Curriculum

Our faculty continues to do an outstanding job of developing and delivering the curriculum. We are extremely grateful for their considerable time and effort in maintaining the high-quality course work that we strive to provide. The Curriculum Development and Integration Committee (CDIC)—Adrian Krainer (Chair), David Jackson, Leemor Joshua-Tor, Nicholas Tonks, and Glenn Turner—continues to carefully monitor and develop the curriculum. In addition to the outstanding course instructors and guest lecturers from within the Laboratory, our courses also continue to attract an impressive array of guest lecturers from other institutions.

New Courses in Quantitative Biology

This year, we developed two new courses in the rapidly emerging field of quantitative biology. Today's science often relies on the use of theoretical modeling and the analysis of vast quantities of data. We therefore need to train scientists who not only are experts in biology, but also have skills in mathematics, physics, chemistry, and computation. The new courses were designed to expand the curriculum to the areas of statistics, programming, bioinformatics, and theoretical modeling and analysis and take different approaches to quantitative thinking. The first course, Physical Biology of the Cell, which was developed by Leemor Joshua-Tor and Rob Phillips from the California Institute of Technology, teaches the students

how to apply concepts from the physics world and how to use computational approaches in looking at biological concepts in a new way. The second course, Quantitative Biology, was developed by faculty member Mickey Atwal to provide students with the tools to interpret large complex data sets and to use statistical methodology in their research. Full descriptions of these courses can be found in the Curriculum section.

Recruiting Efforts

Recruitment for the graduate program's 2010 class and our Undergraduate Research Program (URP) of 2010 was once again managed by Ms. Dawn Pologruto, the School's Director for Admissions and Student Affairs. As in years past, Dawn, our faculty, and our students traveled the length and breadth of the country representing CSHL and WSBS. This year, they were joined by Keisha John who made special recruitment visits aimed at enhancing diversity. The table below details recruitment fairs and conferences in which we have participated, together with the names of faculty, students, and administrators who represented WSBS on these occasions. To further raise awareness of our programs, we also mailed more than 15,000 letters to colleges and universities in the United States and abroad.

A New Website

The WSBS website (http://www.cshl.edu/gradschool) underwent a complete redesign in 2010. This newly enhanced and modernized website describes our unique approach to graduate education and showcases our students, faculty, and alumni through personalized profiles and perspectives. The enhanced site is more user friendly and more visually appealing. Potential students can now link directly to the online Ph.D. program and URP applications and complete their applications online, making it easier to apply and reducing the amount of paper being used. Although still being "tweaked," the new site is already having an impact on our recruitment efforts. The School witnessed an increase of more than 50% in the number of applications received for the entering class of 2011, our second largest pool of applicants since we were founded.

Interinstitutional Academic Interactions

It is important to bear in mind that many of the graduate students who pursue their thesis research at CSHL are not in the WSBS graduate program. Indeed, a large percentage of students are from Stony Brook University (SBU), via a program established between CSHL and SBU more than 30 years ago. In addition, we often have visiting students from institutions around the world who come and do their studies in our laboratories. WSBS provides an on-site "home" for these students, helps to ensure that they feel part of the CSHL community, and assists them with the complexities of performing doctoral research away from their parent institutions. The following students, listed in the box below, joined us this year.

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS		
Student	CSHL research mentor	Affiliation and program
Fatma Bezirci	Adrian R. Krainer	Stony Brook, Molecular and Cellular Biology
Wen Huang	Partha Mitra	Stony Brook, Biomedical Engineering
Huan Qi	Scott Powers	Stony Brook, Applied Math and Statistics
Junwei Shi	Bruce Stillman	Stony Brook, Molecular and Cellular Biology
Guoli Sun	Alexander Krasnitz	Stony Brook, Applied Math and Statistics
Matthew Titmus	Michael Schatz	Stony Brook, Molecular and Cellular Biology
Jason Tucciarone	Z. Josh Huang	Stony Brook, Neuroscience

2010 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS attendees/titles
Hunter College: MARC/RISE Program Visit	Cold Spring Harbor Laboratory	January 14	Dawn Pologruto, Director of Admissions and Student Affairs
Rice University: The Institute of Biosciences and Bioengineering Information Fair	Rice University	January 27	Dawn Pologruto
University of Notre Dame: Career and Internship Fair	University of Notre Dame	February 3	Information sent for distribution
Wellesley College: Women in Science Fair	Wellesley College	February 24	Information sent for distribution
Molloy College: Information Session and Visit	Cold Spring Harbor Laboratory	April 9	Alyson Kass-Eisler, Director of Curriculum; Katie Liberatore, Graduate Student; Hassana Oyibo, Graduate Student; Dawn Pologruto: Amy Rappaport, Graduate Student
Stanford University: Spring Career Fair	Stanford University	April 14–15	Dawn Pologruto
California Forum for Diversity: Graduate School Fair	University of California, Channel Islands	April 17	Dawn Pologruto
Practical Summer Workshop in Functional Genomics: Information Session	The Ohio State University	June 24	Doreen Ware, Associate Professor
New York City College of Technology: NSF STEP Program Visit	Cold Spring Harbor Laboratory	July 15	Dawn Pologruto; Amy Rappaport
University of Medicine and Dentistry, New Jersey: Summer Internship Program Visit	Cold Spring Harbor Laboratory	July 23	Dawn Pologruto; Fred Rollins, Graduate Student
The Protein Society: 24th Annual Symposium and Graduate School Fair	San Diego, California	August 4	Hassana Oyibo
Brookhaven National Laboratory: Graduate School Fair and Annual Symposium	Brookhaven National Laboratory	August 11–12	Keisha John, WSBS Associate
Hispanic Association of Colleges and Universities: 24th Annual Conference	San Diego, California	September 18–20	Hassana Oyibo; Petr Znamenskyiy, Graduate Student
University of Maryland, Baltimore County: Meyerhoff Scholarship Program Visit	University of Maryland, Baltimore County	September 20	Keisha John
The Johns Hopkins University: Information Session	The Johns Hopkins University	September 21	Keisha John
Morgan State University: MBRS-RISE Program Graduate Career Workshop and Information Session	Morgan State University	September 22	Keisha John
Massachusetts Institute of Technology: Career Fair	Massachusetts Institute of Technology	September 23	Amy Rappaport; Jeremy Wilusz, WSBS Alumni
University of Maryland, Eastern Shore: Information Session	University of Maryland, Eastern Shore	September 23	Keisha John
Duke University: Information Session	Duke University	September 23	Ian Peikon, Graduate Student
Massachusetts Institute of Technology: Information Session	Massachusetts Institute of Technology	September 24	Amy Rappaport; Jeremy Wilusz, WSBS Alumni
Big 10+ Graduate School Fair	Purdue University	September 27	Josh Sanders, Graduate Student
Cornell University: Information Session	Cornell University	September 28	Eugene Plavskin, Graduate Student; Petr Znamenskiy
The Johns Hopkins University: Career Fair	The Johns Hopkins University	September 29	Marek Kudla, Graduate Student
Cornell University: Graduate and Professional School Day	Cornell University	September 29	Eugene Plavskin; Petr Znamenskiy
Notre Dame University: Information Session	Notre Dame University	September 30	Kristen Delevich, Graduate Student
Society for Advancement of Chicanos and Native Americans in Science (SACNAS)	Anaheim, California	September 30– October 2	Melanie Eckersley-Maslin, Graduate Student; Keisha John
Hampton University: Information Session	Hampton University	October 4	Keisha John
Hunter College: Minority Access for Research Careers (MARC) Program Information Session	Hunter College	October 6	Hassana Oyibo; Chris Vakoc, CSHL Fellow
Howard University: Research Talk and Information Session	Howard University	October 6	Keisha John; Alea Mills, Associate Professor
University of Puerto Rico, Rio Piedras: Minority Access for Research Careers (MARC) Program Information Session	University of Puerto Rico, Rio Piedras	October 6	Paloma Guzzardo, Graduate Student

2010 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE (*continued*)

Event	Location	Date	WSBS attendees/titles
University of California, Berkeley: Graduate School Fair	University of California, Berkeley	October 6–7	Megan Bodnar, Graduate Student
Oberlin College: Information Session	Oberlin College	October 7	Saya Ebbesen, Graduate Student
Northwestern University: Information Session	Northwestern University	October 7–8	Mitch Bekritsky, Graduate Student
Barry University: Minority Access for Research: Careers (MARC) Program Information Session	Barry University	October 8	Maria Pineda
Washington University, St. Louis: Information Session	Washington University, St. Louis	October 12	Keisha John
University of Wisconsin, Madison: Graduate School Fair	University of Wisconsin, Madison	October 13	Dario Bressan, Graduate Student
Princeton University: Information Session	Princeton University	October 14	Michael Pautler, Graduate Student
University of North Carolina, Chapel Hill: Graduate School Fair	University of North Carolina, Chapel Hill	October 15	Zina Perova, Graduate Student
Princeton University: Graduate and Professional School Fair	Princeton University	October 15	Michael Pautler
Rowan University: Information Session	Rowan University	October 15	Dawn Pologruto
Duke University: Graduate School Fair	Duke University	October 18	Stephen Shea, Assistant Professor
Howard University: Information Session	Howard University	October 19	Keisha John
Queens College, City University of New York: MARC-USTAR Program Information Session	Queens College, City University of New York	October 20	Keisha John
Emory University: Graduate School Fair	Emory University	October 21	Keisha John
Spelman College: MARC-USTAR Program Information Session	Spelman College	October 21	Keisha John
State University of New York, Farmingdale: Information Session and Visit	Cold Spring Harbor Laboratory	October 22	Keisha John; Hassana Oyibo; Michael Pautler; Dawn Pologruto
Temple University: MARC Program: Information Session	Temple University	October 22	Keisha John
California Institute of Technology: Career Fair	California Institute of Technology	October 26	Elizabeth Nakasone, Graduate Student
University of California, Los Angeles: Graduate School Fair	University of California, Los Angeles	October 27	Elizabeth Nakasone
Yale University: Information Session	Yale University	October 28	Kyle Honegger, Graduate Student
University of Southern California: Information Session	University of Southern California	October 28	Elizabeth Nakasone
University of Pittsburgh: Information Session	University of Pittsburgh	October 28	Kristen Delevich
Morehouse College: Graduate School Fair and Research Training Symposium	Morehouse College	October 28–29	Keisha John
Harvard University: Information Session	Harvard University	October 29	Kyle Honegger
Atlanta University Center Consortium: (Morehouse College, Spelman College, and Clark Atlanta University)	Clark Atlanta University	October 29–30	Keisha John
Vassar College: Genetics and Bioinformatics Course Visit and Information Session	Cold Spring Harbor Laboratory	November 1	Keisha John; Justin Kinney, CSHL Fellow; Sheldon McKay; Dawn Pologruto; Amy Rappaport
Annual Biomedical Conference for Minority Students (ABRCMS)	Charlotte, North Carolina	November 10–13	Saya Ebbesen; Paloma Guzzardo; Keisha John; Dawn Pologruto
University of New Mexico: Information Session	University of New Mexico	November 10	Katie Liberatore, Graduate Student
Sigma Xi Annual Conference and Research Symposium	Raleigh, North Carolina	November 11–13	Maria Pineda
American Indian Science and Engineering Society: 2010 National Conference	Albuquerque, New Mexico	November 11–12	Katie Liberatore
Queensborough Community College, CUNY: Cooperative Education Forum	Queensborough Community College, CUNY	November 16	Information sent for distribution
Brooklyn College of CUNY: MARC and RISE Program: Information Session	Brooklyn College of CUNY	November 16	Keisha John
The American Society for Cell Biology Annual Meeting: Graduate School Fair	Philadelphia, Pennsylvania	December 11	Megan Bodnar

Graduate Student Symposium

Continuing the tradition that began in the summer of 2008, three Graduate Student Symposia were held in 2010 at the Laboratory's Genome Research Center in Woodbury: one each in January, May, and October. Each Symposium consisted of a full day of activities including 20-minute and 10-minute talks by senior students and poster presentations by more junior students. Lunch and a wine and cheese reception rounded out the program and provided opportunities for more informal interactions. As with the inaugural event held in August 2008, the students and faculty in attendance found the Symposia to be very worthwhile events. The Graduate Student Symposium is open to the entire Laboratory community, but it is run largely by the students themselves. We are grateful to the two student chairs of the Symposium—Megha Rajaram (SBU) and Maria Pineda (WSBS)—for their dedication and hard work and to Keisha John for overseeing the administrative elements.

Graduate Student and Postdoctoral Fellow Departures

With each year come not only new arrivals, but also departures. The following graduate students and postdoctoral fellows departed from the Laboratory during 2010:

Postdoctoral Fellows

Uli C. Bialucha	Dheeraj Malhotra	Rajesh K. Prakash	Michael Wittinger
Chunlin Cai	Louis Manganas	Vladimir Vacic	Zhiguang Yuchi
Iver K. Hansen	Nael Nadif Kasri	Daniel Valente	Lixing Zhan
Danielle Irvine	Michaela Oswald-Pisarski	Haibin Wang	Zhihua Zhang
Valery Krizhanovsky	Mario Pagani		

Graduate Students

Anne Burlet-Parendel	Jiahao Huang	Shraddha Pai	Rahul Sinha
Sungwook Choi	Mary Kusenda	Benjamin Roland	Shuying Sun
Galen Collins	Lucile Lefevre	Patrick Russell	Oliver Tam
Ashesh Dhawale	Amy Leung	Laurianne Scourzic	Sophie Thomain
Julien Dos Santos	Hiroshi Makino	Grzegorz Sienski	Yang Yang
Ewa Grabowska	Colin Malone	David Simpson	Jianping Zhang
Ikuko Hotta			

Executive Committee

A large measure of the Watson School's success can be traced to the sage advice, guidance, and governance of the School's Executive Committee. I wish to thank faculty members Mickey Atwal, Terri Grodzicker, Adrian Krainer, Alea Mills, David Spector, and Linda Van Aelst who served on the Executive Committee through 2010. Terri Grodzicker completed her second term in December 2010. We are grateful for the insights and advice that Terri brought to the Committee as a member for 6 years. As happens each year, there was also turnover among the student representatives. SBU representative Matthew Lazarus completed his term in October, and he was replaced by Matthew Camiolo. WSBS representative Megan Bodnar also completed her term in December. We are thankful to Matthew and Megan for their honest and thoughtful advice.

Special Events: The Gavin Borden Fellow

The annual Gavin Borden Visiting Fellow (so named after the energetic and charismatic publisher of Molecular Biology of the Cell, who died of cancer in 1991) brings to CSHL an eminent researcher and educator to give the Gavin Borden Lecture, which is dedicated to the graduate students at the

laboratory. Dr. Gerald Joyce, from The Scripps Research Institute, was this year's Gavin Borden Fellow. Dr. Joyce is interested in understanding how Darwinian evolution at the molecular level helps to shape the living world. His current research involves the development of directed molecular evolution techniques and their application to the analysis and design of RNA enzymes. His lecture *The Origin of Life in the Laboratory* was thoroughly enjoyed and provoked many questions from the audience. In addition, the students joined Dr. Joyce during a roundtable lunch and discussion on "What Is Life … and Would You Know It If You Saw It?"

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 2010 programs possible, including The Banbury Fund, The Arnold and Mabel Beckman Foundation, Bristol-Myers Squibb Corporation, Mr. and Mrs. Richard M. Cashin, Mr. and Mrs. Landon Clay, Lester Crown, The Dana Foundation, The William Stamps Farish Fund, The Genentech Foundation, Mr. and Mrs. Alan Goldberg, Florence Gould Foundation, William Randolph Hearst Foundation, Dr. Mark Hoffman, Annette Kade Charitable Trust, Mr. David H. Koch, Mr. and Mrs. Robert D. Lindsay and Family, Mr. and Mrs. David Luke III, Mr. and Mrs. William R. Miller, The Millipore Foundation, OSI Pharmaceuticals Foundation, Mr. and Mrs. John C. Phelan, The Quick Family, The Rathmann Family Foundation, and The Roy J. Zuckerberg Family Foundation.

We are also very fortunate to hold a National Research Service Award Predoctoral Training Grant from the National Institutes of Health, National Institute of General Medical Sciences. The School will be submitting a competitive renewal of this award in early 2011.

Student Achievements

The WSBS students continue to impress us all with their accomplishments. They publish their research findings in prestigious international journals and obtain fellowships to pursue their research interests. Our students have published more than 170 papers to date, a remarkable accomplishment for a School in its 12th year. In addition, our current students and alumni have been successful in receiving prestigious awards and fellowships. In 2010, Katie Liberatore was awarded a National Science Foundation Graduate Research Fellowship. She was also selected to attend the PepsiCo Company Day at the New York Academy of Sciences. Elizabeth Nakasone was awarded a U.S. Army Medical Research and Materiel Command Breast Cancer Research Program Predoctoral Fellowship. Amy Rappaport was selected to present at the 5th annual "Cracking the Code with the Bear" Research Symposium. Elvin Wagenblast was awarded a predoctoral fellowship from the Boehringer Ingelheim Fonds. Recent graduate Yaniv Erlich was awarded the prestigious Harold M. Weintraub International Award for Graduate Studies. He was also named a 2010 Genome Technology Young Investigator. Graduate Christopher Harvey was named the 2010 Larry Katz Memorial Lecturer for the CSHL meeting on Neuronal Circuits. Graduate Colin Malone received a Helen Hay Whitney Postdoctoral Fellowship, and graduate Jeremy Wilusz received a Leukemia and Lymphoma Society Postdoctoral Fellowship.

Alumni in Faculty and Senior Positions

To date, 45 students have received their Ph.D. degrees from the WSBS. Of these graduates, eight currently hold tenure-track faculty positions and two hold independent research positions. Twenty-seven of our former students are pursuing postdoctoral research and five of our alumni completed their postdoctoral studies and have moved onto positions in administration, management consulting, and journal editing. We are so proud of them all!

WSBS GRADUATES IN FACULTY AND SENIOR POSITIONS

Name	Current position
François Bolduc	Assistant Professor, University of Alberta, Canada
Darren Burgess	Assistant Editor, *Nature Reviews Cancer* and *Nature Reviews Genetics*, UK
Amy Caudy	Assistant Professor, University of Toronto, Canada
Catherine Cormier	Scientific Liaison, Arizona State University
Yaniv Erlich	Whitehead Fellow, Whitehead Institute
Rebecca Ewald	Project Manager, Roche Diagnostics, Inc.
Elena Ezhkova	Assistant Professor, Developmental and Regenerative Biology, Mount Sinai School of Medicine
Ira Hall	Assistant Professor, Biochemistry and Molecular Genetics, University of Virginia School of Medicine
Keisha John	WSBS Associate
Zachary Lippman	Assistant Professor, Cold Spring Harbor Laboratory
Patrick Paddison	Assistant Member, Human Biology Division, Fred Hutchinson Cancer Research Center
Emiliano Rial-Verde	Engagement Manager, McKinsey & Co., Inc., Geneva, Switzerland
Ji-Joon Song	Assistant Professor, Biological Science, Korea Advanced Institute of Science and Technology (KAIST), South Korea
Niraj Tolia	Assistant Professor, Molecular Microbiology, School of Medicine at Washington University, St. Louis

2010 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS

Blum A, Dubnau JT. 2010. Parallel processing of olfactory memories in *Drosophila. Fly* (Austin) 4: 163–166.

Bolduc FV, Bell K., Rosenfelt C, Cox H, Tully T. 2010. Fragile X mental retardation 1 and filamin a interact genetically in *Drosophila* long-term memory. *Front Neural Circuits* 3: 22.

Chicas A, Wang X, Zhang C, McCurrach M, Zhao Z, **Mert O,** Dickins RA, Narita M, Zhang M, Lowe SW. 2010. Dissecting the unique role of the retinoblastoma tumor suppressor during cellular senescence. *Cancer Cell* 17: 311–414.

Chiu SL, Cline HT. 2010. Insulin receptor signaling in the development of neuronal structure and function. *Neural Dev* 5: 7.

Collins GA, Gomez TA, Deshaies RJ, Tansey WP. 2010. Combined chemical and genetic approach to inhibit proteolysis by the proteasome. *Yeast* 27: 965–974.

Edvardson S, Shaag A, Zenvirt S, **Erlich Y**, Hannon GJ, Shanske AL, Gomori JM, Ekstein J, Elpeleg O. 2010. Joubert syndrome 2 (JBTS2) in Ashkenazi Jews is associated with a TMEM216 mutation. *Am J Hum Genet* 86: 93–97.

Egeblad M, **Nakasone ES,** Werb Z. 2010. Tumors as organs: Complex tissues that interface with the entire organism. *Dev Cell* 18: 884–901.

Erlich Y, Gordon A, Brand M, Hannon GJ, Mitra PP. 2010. Compressed genotyping. *IEEE Trans Inf Theory* 56: 706–723.

Haase AD, **Fenoglio S,** Muerdter F, **Guzzardo PM,** Czech B, Pappin DJ, Chen C, Gordon A, Hannon GJ. 2010. Probing the initiation and effector phases of the somatic piRNA pathway in *Drosophila. Genes Dev* 24: 2499–2504.

Li J, Wang Y, **Chiu SL,** Cline HT. 2010. Membrane targeted horseradish peroxidase as a marker for correlative fluorescence and electron microscopy studies. *Front Neural Circuits* 26: 6.

Malone CD, Hannon GJ. 2010. Molecular evolution of piRNA and transposon control pathways in *Drosophila. Cold Spring Harbor Symp Quant Biol* 74: 225–234.

Mangone M, Myers MP, Herr W. 2010. Role of the HCF-1 basic region in sustaining cell proliferation. *PLoS One* 5: e9020.

Mavrakis KJ, Wolfe AL, Oricchio E, Palomero T, de Keersmaecker K, **McJunkin K,** Zuber J, James T, Khan AA, Leslie CS, Parker JS, Paddison PJ, Tam W, Ferrando A, Wendel HG. 2010. Genome-wide RNA-mediated interference screen identifies miR-19 targets in Notch-induced T-cell acute lymphoblastic leukaemia. *Nat Cell Biol* 12: 372–379.

Pineau P, Volinia S, **McJunkin K,** Marchio A, Battiston C, Terris B, Mazzaferro V, Lowe SW, Croce CM, Dejean A. 2010. miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci* 107: 264–269.

Wei W, Nguyen LN, Kessels HW, Hagiwara H, Sisodia S, Malinow R. 2010. Amyloid β from axons and dendrites reduces local spine number and plasticity. *Nat Neurosci* 13: 190–196.

Wilusz JE, Spector DL. 2010. An unexpected ending: Noncanonical 3′ end processing mechanisms. *RNA* 16: 259–266.

Yao Z, **Fenoglio S,** Gao DC, Camiolo M, Stiles B, Lindsted T, Schlederer M, Johns C, Altorki N, Mittal V, Kenner L, Sordella R. 2010. TGF-β IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer. *Proc Natl Acad Sci* 107: 15535–15540.

WSBS Family Events

Finally, I am pleased to announce that this was a great year for the WSBS students as we celebrated some wonderful personal occasions. On April 24, Sang Geol Koh, from the entering class of 2008, and his wife Bomi welcomed a son, Aiden, who weighed in at 8 lbs 11 oz. On May 30, Katie Liberatore, also from the class of 2008, married her fiancée Brian Kramer in New Mexico. On June 21, Kyle Honegger from the entering class of 2007 married Laura Bozue in their home state of Illinois. Silvia Fenoglio from the entering class of 2009 married postdoctoral fellow Agustin Chicas on July 18. Silvia and Agustin welcomed their daughter Lucia on December 22. Our congratulations and best wishes to all of them.

Leemor Joshua-Tor
Professor and Dean

Kyle and Laura

Katie and Brian

Silvia and Agustin

Aiden Koh

Lucia Chicas

SPRING CURRICULUM

Topics in Biology

ARRANGED BY   **Leemor Joshua-Tor, Alyson Kass-Eisler, and Jan A. Witkowski**

FUNDED IN PART BY   **The Daniel E. Koshland, Jr. Visiting Lectureship; The David Pall Visiting Lectureship; The Fairchild Martindale Visiting Lectureship; The Lucy and Mark Ptashne Visiting Lectureship; The Michel David-Weill Visiting Lectureship**

Each year, one or a team of invited instructors offer 7-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In Spring 2010, there were two such courses: a new course on Physical Biology of the Cell and Fundamental Concepts in Statistics.

Physical Biology of the Cell

Attended by the entering classes of 2006 and 2009

INSTRUCTORS   **Rob Phillips,** California Institute of Technology
                     Hernan Garcia, California Institute of Technology
                     Ron Milo, Weizmann Institute, Israel
                     Julie Theriot, Stanford University
                     Kerwyn Huang, Stanford University
                     Daniel Fisher, Stanford University
                     Jane Kondev, Brandeis University

VISITING LECTURERS   **Daniel Fisher,** Stanford University
                     Kerwyn Huang, Stanford University
                     Jané Kondev, Brandeis University
                     Ron Milo, Weizmann Institute, Israel
                     Julie Theriot, Stanford University

TEACHING FELLOW   **Hernan Garcia,** California Institute of Technology

The aim of this course was to provide a hands-on experience in the use of quantitative models as a way to view biological problems. We began with "order of magnitude biology," showing how simple estimates can be exploited in biology. We 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 which are nearby in physical biology seem to be unrelated when viewed from the vantage point of molecular or cell biology. The instructors guided the students from start to

Physical Biology of the Cell course participants: (*Top row*) Wee Siong Goh, Jané Kondev, Ian Peikon, Hassana Oyibo, Daniel Fisher, Felix Schlesinger, Kristen Delevich, Rob Phillips, Hernan Garcia, Julie Theriot, Leemor Joshua-Tor; (*middle row*) Saya Ebbesen, Megan Bodnar, Melanie Eckersley-Maslin, Cinthya Zepeda Mendoza, Amy Rappaport, Stephane Castel; (*bottom row*) Claudio Scuoppo, Silvia Fenoglio, Yaniv Erlich, Ron Milo.

finish on several modeling case studies. The course ran from Saturday, March 21, to Sunday, March 28, and was one of the most interesting (and fun) but challenging courses the students have taken.

Fundamental Concepts in Statistics

Attended by the entering classes of 2007 and 2008

INSTRUCTOR	**Martina Bremmer,** San Jose State University
VISITING LECTURERS	**Robert Martienssen,** Cold Spring Harbor Laboratory
	Madhu Mazumdar, Weill Cornell Medical College
TEACHING FELLOWS	**Tilman Achberger,** Purdue University
	Gayla Olbricht, Purdue University

Statistics and mathematics have become an integral part of research in many areas of biology. New technology leads to large amounts of data produced in the fields of genomics, ecology, and epidemiology. The statistical methods required to analyze the data evolve and adapt constantly with the changing demands. However, the underlying principles of statistical analysis remain the same.

This course focused on the fundamental statistical concepts used in the analysis of biological data. Emphasis was placed on statistical reasoning, rather than specific formulas and computations. However, examples of statistical applications were discussed and students were asked to apply the methods they learned to real data sets using software. The major statistical concepts introduced in this course included descriptive statistics, statistical inference, sampling and sampling distributions, correlation, hypothesis testing, experimental design, regression, and ANOVA. The course integrated lectures by the instructor with directed reading and interpretation of statistical analyses in publications by the

Fundamental Concepts in Statistics course participants: (*Front row*) Paloma Guzzardo, Eugene Plavskin, Katie Liberatore, Beth Nakasone, Carrie Clendaniel, Susann Weissmueller, Ozlem Mert, Martina Bremer, Megan Bodnar, Melanie Eckerley-Maslin, Nilgun Tasdemir; (*back row*) Josh Sanders, Saya Ebbesen, Peter Znamenskiy, Doug Baumann, Hassana Oyibo, Sang-Geol Koh, Philippe Batut, Elvin Wagenblast, Molly Moscoe, Marek Kudla, Joe Calarco, Zhenxun Wang, Kyle Honegger, Maria Pineda, Jiahao Huang, Zina Perova.

students. It was accompanied by lab sections in which the students were instructed on conducting simple statistical analyses using software. In addition, invited speakers covered special topics in the application of statistical methodology to the biological sciences. The course ran from Sunday to Saturday, March 28–April 3, and was organized and largely taught by Martina Bremmer. Overall, the students thought it was a great course and one of the most useful for their own research.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR	David A. Micklos	
INSTRUCTORS	Amanda McBrien (Lead)	Jermel Watkins
	Jennifer Galasso	Elna Gottlieb
	Jason Williams	Erin McKechnie
ADMINISTRATOR	Carolyn Reid	

As science plays an increasing role in society, there is also an increasing need for biologists to educate nonscientists of all ages about biology. The Watson School of Biological Sciences doctoral program offers its students unique teaching experiences through the Laboratory's Dolan DNA Learning Center, where students teach laboratory courses to high school and middle school students. From these teaching experiences, they learn how to communicate with nonbiologists and to inspire and educate creative young minds. The teaching module entailed pairs of students teaching one morning or afternoon a week for 12 weeks. In the initial weeks, the Dolan DNA Learning Center instructors

taught the Watson School students the didactic process; it was not until the fifth week that the graduate students taught on their own. At the end of the 12 weeks, the students were very excited about their teaching experience.

Laboratory Rotations

The most important element of a doctoral education is learning to perform independent research that leads to a unique contribution to human knowledge. After the fall course term, students participate in laboratory rotations. These rotations provide students and faculty the opportunity to get to know each other and to explore possibilities for doctoral thesis research. At the end of each rotation, students make short presentations of their studies to the other students and their rotation advisors. These talks give students an opportunity to share their laboratory experiences and to learn how to give a scientific presentation. With this latter goal in mind, in addition to the research mentors, the instructors of the Scientific Exposition and Ethics core course and members of the School's Executive Committee attend the talks and give individual feedback to students on their presentations. This year, 13 WSBS faculty members and one CSHL fellow served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Josh Dubnau	Bo Li	Bruce Stillman
Gregory Hannon	Robert Martienssen	Marja Timmermans
Z. Josh Huang	Raffaella Sordella	Christopher Vakoc
David Jackson	David L. Spector	Linda Van Aelst
Adam Kepecs		

FALL CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

INSTRUCTORS	**Gregory Hannon (Lead)**	**Leemor Joshua-Tor**
	Alexander Gann	**Zachary Lippman**
	Z. Josh Huang	**Nicholas Tonks**
GUEST LECTURERS	**Josh Dubnau**	**Robert Martienssen**
	Mikala Egeblad	**Thomas Schalch (postdoctoral fellow)**
	David Jackson	
VISITING LECTURER	**Tracy Bale,** University of Pennsylvania	

The SRL core course was revamped this year with respect to content and instructor participation. Each module of the course has now been designed to cover a different general theme and proceeds with the goal of considering an open, still unanswered, scientific question. In addition, the first two modules of the course were combined into one longer module so that an integrated theme could be tackled using two different approaches: molecular biology and structural biology.

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the curriculum, students (1) acquire a broad base of knowledge about the biological sciences, (2) learn the scientific method, and (3) learn how to think critically. The beginning of the course was divided into four to five modules, each of which had a different general theme, and proceeded with the goal of considering an open, still unanswered, scientific question. For each module, students read an assigned set of research articles (generally four articles) and at the end of the module, they provided written answers to a problem set that guides 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. During each week, the students met among themselves to discuss the assigned papers not covered by the problem set. Each week, students spent an evening discussing the assigned articles with faculty. In the final part of the course, students participated in a mock study section in which real National Institutes of Health, R01 grants were reviewed and critiqued. This allows the students to evaluate the questions before the answers are known, evaluate routes toward discovery before knowing where they will end, and make critical judgments about how to proceed in the face of an uncertain outcome.

The module topics for this course were as follows:

Topic	Instructor(s)
Mechanism and Structure of Gene Regulation	Alex Gann
	Leemor Joshua-Tor
Developmental Genetics and Morphological Diversity	Zachary Lippman
Signal Transduction and the Search for Novel Therapeutics	Nicholas Tonks
The Brain: Does Chromatin Matter?	Z. Josh Huang
Study Section	Gregory Hannon

The Darrell Core Course on Scientific Exposition and Ethics

INSTRUCTORS	**David J. Stewart (Lead)**
	Alea A. Mills
	Arne Stenlund
TEACHING ASSISTANT	**Jonathan Ipsaro**
GUEST LECTURERS	**Walter Goldschmidts**
	Jason Wen
	Jan A. Witkowski
VISITING LECTURERS	**Olga Akselrod,** The Innocence Project
	Keith Baggerly, University of Texas, M.D. Anderson Cancer Center
	Robert Charrow, Greenberg Traurig, LLC
	Avner Hershlag, North Shore University Hospital
	Amy Harmon, *The New York Times*
	Boyana Konforti, Editor, *Nature Structural & Molecular Biology*
	Paul Offit, Children's Hospital of Philadelphia
	Joy Simha, Young Survivors Coalition, National Breast Cancer Coalition
	Liz Wohl, National Breast Cancer Coalition

The Scientific Exposition and Ethics (SEE) core course in 2010 was taught by the same instructor team as last year, with David Stewart taking the lead role, joined by Alea Mills and Arne Stenlund. This year, CSHL postdoctoral fellow Jonathan Ipsaro participated in the course as a teaching assistant. As usual, the course hosted expert guest lecturers who covered topics that included scientific presentations, the editorial review process, ethical and legal responsibilities of scientists, DNA profiling, and postconviction appeals, patient advocacy, and reproductive genetics.

This core course offered instruction in the fundamental elements of scientific exposition—writing skills and public speaking—and ethics. The ability to communicate effectively and to appreciate the intricacies of ethical issues are essential skills for biologists; both subjects were taught in a series of example-based lectures and discussion groups. Writing skills included the fundamentals of modern scientific English and the organization and preparation of papers, research abstracts, and grant applications. Oral presentation skills were taught by instructors with different modes of presentation. Together with instructors, students critiqued formal seminar presentations at the Laboratory. Instruction and discussions about ethics included the ethical implications of biological discovery on society as well as the nature and boundaries of ethical behavior of scientists and their rights and responsibilities. A primary objective of the course was that students consider exposition and ethics integral aspects of scientific research.

Research Topics

ORGANIZERS	**Kimberley Geer**
	Alyson Kass-Eisler

This core course provided students with an in-depth introduction to the fields of research that the Laboratory scientists investigate. Students and faculty attended a weekly Research Topics seminar, at which faculty members and CSHL fellows presented their current research topics and methods

of investigation each Wednesday evening over dinner. The students learned how to approach important problems in biology. These seminars, together with the annual fall Laboratory In-House Symposium, provided students with a basis for selecting laboratories in which to do rotations. The weekly speakers are listed below:

Florin Albeanu	David Jackson	Alea A. Mills	Nicholas Tonks
Gurinder (Mickey) Atwal	Leemor Joshua-Tor	Partha Mitra	Lloyd Trotman
Anne Churchland	Adam Kepecs	Pavel Osten	Glenn Turner
Josh Dubnau	Alexei Koulakov	Darryl Pappin	Christopher Vakoc
Mikala Egeblad	Adrian R. Krainer	Michael Schatz	Linda Van Aelst
Grigori Enikolopov	Alexander Krasnitz	Stephen Shea	Doreen Ware
Hiro Furukawa	Bo Li	Raffaella Sordella	Michael Wigler
Thomas Gingeras	Zachary Lippman	David L. Spector	Anthony Zador
Christopher Hammell	Scott Lowe	Arne Stenlund	Hongwu Zheng
Gregory Hannon	Robert Martienssen	Bruce Stillman	Yi Zhong
Z. Josh Huang	W. Richard McCombie	Marja Timmermans	

Specialized Disciplines Courses

A new Specialized Disciplines course in *Quantitative Biology* was developed by Mickey Atwal to provide the students with the tools to interpret large, complex data sets and to use statistical methodology in their research. It is one of two new courses in the areas of statistics, programming, bioinformatics, and theoretical modeling and analysis that the School developed in 2010 to complement our existing curriculum. With the addition of this course, the students in the entering class of 2010 took a total of four Specialized Disciplines courses this fall.

The other three Specialized Disciplines courses are *Cellular Structure and Function, Genetics and Genomics*, and *Systems Neuroscience. Cellular Structure and Function* was again taught by lead instructor Linda van Aelst and Raffaella Sordella, but ran as a week-long intensive course when the students first arrived. Josh Dubnau led *Genetics and Genomics* and was joined again by Thomas Gingeras. Anthony Zador led the *Systems Neuroscience* course, assisted by Adam Kepecs and Glenn Turner.

Cellular Structure and Function

INSTRUCTORS	**Linda Van Aelst (Lead)**
	Raffaella Sordella
GUEST LECTURERS	**Darryl Pappin**
	David L. Spector
	Bruce Stillman
VISITING LECTURERS	**Gregg Gundersen,** Columbia University
	Aron Jaffe, Novartis
	Alexey Khodjakov, Wadsworth Laboratory
	Sandy Simon, The Rockefeller University
	Marc Symons, The Feinstein Institute for Medical Research

With the complete genome sequence available for many organisms, there is now an increasing emphasis on understanding the function of the gene products. This understanding requires an increasing ap-

preciation of the structure and function of the cell as well as dynamic associations within the cell. This course provided a basic overview of the structural and functional organization of cells with particular emphasis on cellular compartmentalization and communication. Topics of focus included the cytoskeleton, cell adhesion and signaling, membrane transport, gene expression, and nuclear organization. In addition, the course provided insight into the basic toolbox of the cell biologist of the 21st century.

Genetics and Genomics

INSTRUCTORS	**Josh Dubnau (Lead)**
	Thomas Gingeras
GUEST LECTURER	**Christopher Hammell**
VISITING LECTURERS	**Hopi Hoekstra.** Harvard University
	Tim Tully, Dart Neuroscience

This course placed modern human genetics and genomics into the context of classical organismal genetics. History, perspective, and technique were described around four levels of analysis: naturally occurring variation, genome evolution, genetic screens, and gene epistasis. How do gene mutations help to define biological processes? How are more complex traits genetically dissected into simpler (underlying) components? What concepts and techniques are used to organize genes into pathways and networks? How are genes mapped, cloned, and engineered to identify functional domains of proteins? What gene variation exists in natural populations? What are the functional consequences of gene variation? How is it detected? How are genomes organized and coordinately regulated? How can genomic information be cataloged, organized, and mined? These questions and concepts were fleshed out using examples from the literature.

Quantitative Biology

INSTRUCTORS	**Mickey Atwal (Lead)**
	Michael Schatz
GUEST INSTRUCTORS	**Justin Kinney (CSHL Fellow)**
	Alexander Krasnitz

With the advent of high-throughput technologies in biology, it has become necessary for biological researchers to be able to analyze and interpret high-dimensional data and frame hypotheses mathematically. To this end, the aim of this course was to equip the students with a basic training in modern statistical methods. By the end of the course, the students were able not only to answer many of the simple statistical questions that arise in data analyses, but also to became familiar with the more complex techniques used by fellow computational biologists. Topics included error fluctuations, calculating the significance of an experimental result, Bayesian inference, information theory, power calculations, dimensional reduction, and DNA sequence analyses. In addition, this course introduced mathematical modeling, motivated by the classic examples in quantitative biology such as the Delbrück-Luria experiment, Hopfield's kinetic proofreading, and Kimura's neutral theory of population genetics.

Systems Neuroscience

INSTRUCTORS   **Anthony Zador (Lead)**
                           Adam Kepecs
                           Glenn Turner

GUEST INSTRUCTORS   **Florin Albeanu**
                           Alexei Koulakov
                           Stephen Shea

This course provided an overview of key aspects neuroscience. The emphasis was on spanning levels: How can we go from molecules through cells and circuits to behavior?

There were three main components to the class: lectures, problem sets, and a final project. The last week of class was spent on the final project in which the students found and presented a neuroscience paper which spanned levels, for example, from the molecular to the synaptic or the circuit level to the behavioral.

POSTDOCTORAL PROGRAM

PROGRAM DIRECTOR   **Nicholas Tonks**

PROGRAM ADMINISTRATOR   **Alyson Kass-Eisler**

Cold Spring Harbor Laboratory (CSHL) is proud of its rich tradition in postdoctoral education and has a deep commitment to the postdoctoral experience. CSHL has long been recognized as a place for nurturing young scientists, with postdoctoral researchers being an integral part of the discovery process. The Laboratory currently employs ~160 postdoctoral fellows working in the labs of 47 principal investigators. Current fields of research expertise include genetics; molecular, cellular, and structural biology; neuroscience; cancer; plant biology; genomics; and quantitative biology. The postdoctoral community at CSHL is diverse and international, arriving at the Laboratory with many different backgrounds and with needs that change during the course of the postdoctoral years. The Postdoctoral Program Office works closely with the fellows and the Laboratory's administration to coordinate and organize educational and career development activities. Dr. Alyson Kass-Eisler, the Postdoctoral Program Officer, and Dr. Nicholas Tonks, the Scientific Director of the Postdoctoral Program, provide the day-to-day support for the postdoctoral program, which is overseen by the Dean of the Watson School, Dr. Leemor Joshua-Tor.

In 2010, we held a number of courses and workshops aimed at postdoctoral fellows. On December 9, the Office of Sponsored Programs, the Development Department, and the Postdoc Program Office held a very popular course on Grant Writing. The sessions included Introduction to Grants and External Funding, Human Subjects/Tissue use in Research, The Grant Abstract—Do's, Don'ts, Peer Review Panel Discussion, Effective Writing Skills, Fellowships, Career Development Awards, and Resources for Postdocs, and One-on-One Consultations.

CSHL has been a member of a special ambassador recruitment program from Merck Research Laboratories (MRL) since 2005. This program provides the postdoctoral fellows and students at CSHL an inside connection to a scientist working at MRL. As a result of this program, Dr. Maarten Hoek, a postdoctoral fellow from Dr. Bruce Stillman's laboratory, was hired as a Research Scientist in 2007 and Dr. Zuo Zhang, a postdoctoral fellow from Dr. Adrian Krainer's laboratory, joined MRL as a Research Scientist in January 2009. Drs. Li and Hoek visited the laboratory again on January 15 for one-on-one recruitment session. In addition, Dr. Li gave a talk on Identification of Glucose-Dependant Insulin Secretion Targets in Pancreatic β Cells by Combining Defined-Mechanism Compound Library Screening and siRNA Gene Silencing, to give the fellows a feel for the type of research being done in industry.

Leadership Training was held April 7 for postdocs interested in planning for their next career step. Sessions included Mentoring Competencies for Success, Excellence Starts with Recruiting High-Level Performers, Selecting A-Players—Right People Doing Right Things, Staffing on Three Interfaces—Science, You, and the Chemistry with Your Staff, Behavior-Based Interviewing Guidelines, Defining Your Team—Providing Direction, and Setting and Managing Expectations.

In the afternoon session on Individual Development Plans (IDP) for Postdoctoral Fellows, Dr. Ginsburg talked about creating an IDP and how the IDP can be a valuable tool for career development. Stemming from this session, a group of postdoctoral fellows started their own "IDP" group to meet monthly to discuss their career goals and plans to achieve them. The second annual National Postdoc Appreciation Day (which this year was changed to National Postdoc Appreciation Week) was held during the week of September 20. To celebrate, CSHL hosted an outdoor pizza party and

a job-searching roundtable discussion. It was a nice opportunity for the Lab to say thank you for all that the postdocs do.

Five years ago, the School initiated a prize for the best posters by a postdoctoral fellow and the best poster by a graduate student at the Laboratory's annual In-House Symposium. Each "prize" is given in the form of an educational grant of $500. In addition to providing a forum for the postdoctoral fellows to show off their research, and potentially win a prize, it gives the entire scientific community a chance to come together and share ideas beyond the wall of their individual laboratories. It has been a great success for both the presenters and the laboratory community as a whole. Seventeen posters were presented for the postdoctoral prize, which was shared this year by Fedor Karginov, a postdoctoral fellow in Greg Hannon's laboratory, and Navasona Krishnan in Nick Tonk's laboratory.

During the past couple of years, we have increasingly widened the role of postdoctoral fellows in the curricular activities of the School. Our fellows have been involved in preparing our students for the challenging Fall Term curriculum by working as one-on-one tutors during the summer. Throughout the Fall Term, postdoctoral fellows are used as tutors for all aspects of the curriculum, providing one-on-one tutoring, group tutoring, and participating in discussion sessions. In 2007, the Scientific Exposition and Ethics course took further advantage of the expertise of our postdoctoral community by hiring two fellows as teaching assistants (TAs). These two fellows were an integral part of the course by providing their expertise in discussions, editing of the students writing work, and critiquing oral presentations. This format was highly successful and was used again in Fall 2010. Jon Ipsaro, a postdoctoral fellow from Leemor Joshua-Tor's laboratory, was this year's TA.

Since 2003, all postdoctoral fellows and graduate students at the Laboratory have been enrolled in a special initiative of the New York Academy of Science (NYAS), called the Science Alliance. The Science Alliance for graduate students and postdoctoral fellows is a consortium of universities, teaching hospitals, and independent research facilities in the New York City metropolitan area that have formed a partnership with NYAS. The aim of the Alliance is to provide career and professional development monitoring for postdoctoral fellows and graduate students in science and engineering, through a series of live events and a dedicated web portal. In addition, the Science Alliance gives graduate students and postdoctoral fellows the opportunity to network with their peers across institutions and with key leaders in academia and industry. This year, the Science Alliance meetings and workshops included The Art of Networking for Science Professionals, Evaluating and Negotiating Job Offers: Demystifying the Process, Going on the Academic Market: Strategies for Scientists, and Writing for Biomedical Publication. In addition, in June of this year, Alyson Kass-Eisler and Dr. Keisha John attended a roundtable discussion with other local leaders in postdoctoral training. They took part in a Workshop on Improvisation for Creativity, Collaboration, and Communication in the Workplace and participated in breakout sessions on Career Development Programming: Addressing Issues and Sharing Ideas for Future Initiatives.

This year, the Laboratory applied to the National Institutes of Health for a postdoctoral training program in cancer biology. The primary goal of the Cancer Gene Discovery and Cancer Biology Postdoctoral Training Program is to prepare the next generation of cancer biologists to pursue independent research that integrates cancer gene discovery with functional biology in order to facilitate the development of novel cancer therapeutic, diagnostic, and/or preventive strategies. We are very pleased to report that the application has been recommended for funding by the review panels.

Finally, a most important measure of our postdoctoral program's success is the ability of postdoctoral fellows to secure positions at the end of their training. In 2010, the Laboratory's departing postdoctoral fellows went on to positions at Albert Einstein College of Medicine; Cold Spring Harbor Laboratory; Donders Centre for Neurosciences, The Netherlands; North Shore University Hospital; Mt. Sinai School of Medicine, N.Y.; Murdoch Children's Research Institute, Melbourne, Australia; University of Buenos Aires, Argentina; University of Texas, Austin; U.S. Army Corps of Engineers; Engineer Research and Development Center; and Weizmann Institute of Science, Israel.

UNDERGRADUATE RESEARCH PROGRAM

PROGRAM DIRECTOR   **Zachary Lippman**

PROGRAM ADMINISTRATOR   **Dawn Pologruto**

The fundamental objective of this program is to give students an opportunity to conduct first-rate research. Participants learn about scientific reasoning, laboratory methods, theoretical principles, and scientific communication. The specific objectives of the program are to (1) give college undergraduates a taste of conducting original research at the cutting edge of science, (2) encourage awareness of the physical and intellectual tools necessary for modern biological research, (3) foster an awareness of the major questions currently under investigation in the biomedical and life sciences, and (4) promote interactions with laboratory scientists through an immersion in the research environment.

During the 10-week program, URPs work with CSHL senior staff members on independent research projects, specifically in the areas of cancer biology, neuroscience, plant biology, cellular and molecular biology, genetics, macromolecular structure, and bioinformatics.

URPs work, live, eat, and play among CSHL scientists. They have a very busy academic and social calendar for the summer. They attend lectures in the Goldberg Faculty Lecture Series from CSHL faculty members and outside faculty members including, in 2010, talks by Dr. Stanley Maloy, San Diego State University, and Dr. Alfred Goldberg, Harvard University. URPs also attend a Bioinformatics Workshop Series, where they learn how to identify patterns in DNA and protein sequences and how to interpret them. Lectures by CSHL faculty are specifically designed for URPs. URPs were also invited to join Dr. and Mrs. Watson for a pizza party and Dr. and Mrs. Stillman for dinner. BBQ and pool parties, volleyball games, sailing lessons, designing the URP T-shirt, competing in the annual CSHL Petri Dish Race and Scavenger Hunt, and the ever-famous URP versus PI volleyball match rounded out the engaging program. 2010 was the second year the URPs beat the faculty team in volleyball—quite an accomplishment!

At the beginning of the summer, URPs write an abstract and present a talk on their proposed research. Concluding the program in August, URPs prepare a final report and present their results in a 15-minute talk at the URP Symposium. The following students, selected from 720 applicants, took part in the 2010 program:

Robert Aboukhalil, McGill University
Advisor: **Dr. Mickey Atwal**
Sponsor: Robert H.P Olney Fund Fellowship
Colocalization of tumor suppressor genes.

Emily Bottle, University of Cambridge
Advisor: **Dr. Anthony Zador**
Sponsor: Former URP Fund Fellowship
Screening the brain areas involved in sensorimotor association.

Alexandra Bryson, Texas A&M University
Advisor: **Dr. Bruce Stillman**
Sponsor: Former URP Fund Fellowship
Exogenous expression of DDX5 RNA helicase.

Diana Cai, Columbia College
Advisor: **Dr. Yi Zhong**
Sponsor: Former URP Fund Fellowship
Combining two gene-targeting systems to investigate cross-talk between the mushroom body and central complex in *Drosophila.*

Joseph Cammarata, Hunter College
Advisor: **Dr. Zach Lippman**
Sponsor: Hunter College Scholar and National Science Foundation Scholar
Searching for a marker of meristem reiteration in *Solanum lycopersicum.*

Jonathan Coravos, Bowdoin College
Advisor: **Dr. Josh Dubnau**
Sponsor: National Science Foundation Scholar
Are dopamine receptors expressed in *Drosophila* glia?

Helen Cha, Williams College
Advisor: **Dr. Marja Timmermans**
Sponsor: National Science Foundation Scholar
The effect of a small RNA gradient on sharpening the adaxial-abaxial boundary in *Arabidopsis thaliana*.

Tiffany Coupet, Johns Hopkins University
Advisor: **Dr. Scott Powers**
Sponsor: William Townsend Porter Foundation Fellowship
Exploring synergistic interactions with RNAi in combination with a PI3K inhibitor.

Martin Fan, Washington University, St. Louis
Advisor: **Dr. Adrian R. Krainer**
Sponsor: Former URP Fund Fellowship
Characterizing the tumorigenic potential of several splicing factors.

Nisha Hariharan, University of California, Berkeley
Advisor: **Dr. David Jackson**
Sponsor: Former URP Fund Fellowship
Cell-to-cell trafficking via plasmodesmata in *Arabidopsis thaliana*.

Ruilong Hu, Washington University, St. Louis
Advisor: **Dr. Stephen Shea**
Sponsor: URP Fund Fellowship
The mechanism of neural selectivity for pup isolation calls in the mouse.

Edward Larkin, University of Notre Dame
Advisor: **Dr. Z. Josh Huang**
Sponsor: University of Notre Dame URP Scholar
The birth and development of cortical chandelier cells.

Diana LaScala-Gruenewald, Massachusetts Institute of Technology
Advisor: **Dr. Michael Q. Zhang**
Sponsor: 30th Anniversary URP Scholar
An interactive genomic map between budding yeast species for the study of DNA replication,

Ashley Maceli, Suffolk University
Advisor: **Dr. Gregory Hannon**
Sponsor: James D. Watson Fellowship
Mammalian genomic simplification methods for studying DNA methylation.

Connie Martin, University of California, Riverside
Advisor: **Dr. Gregory Hannon**
Sponsor: Howard Hughes Medical Institute Scholar
Transposon insertion profiling.

Meg McCue, Dartmouth College
Advisor: **Dr. Partha Mitra**
Sponsor: Former URP Fund Fellowship
The brain architecture project: Quantitative image analysis.

Matthias Minderer, University of Cambridge
Advisor: **Dr. Lloyd Trotman**
Sponsor: Former URP Fund Fellowship
The nuclear import mechanism of PTEN.

The URPs show off their class T-shirts.

Luis Montano, Universidad Nacional Autonoma de Mexico
Advisor: **Dr. W. Richard McCombie**
Sponsor: Former URP Fund Scholar
Solution-based exome capture: Is it useful to detect human variation?

Claudio Morales-Perez, University of Puerto Rico
Advisor: **Dr. Hiro Furukawa**
Sponsor: National Science Foundation Fellowship
Understanding the molecular mechanism of antagonism in the NMDA receptor.

Angelina Regua, Molloy College
Advisor: **Dr. Leemor Joshua-Tor**
Sponsor: National Science Foundation Fellowship
Organization of ClrC (Clr4-Rik1-Cul4) complex.

Hanna Retallack, Harvard University
Advisor: **Dr. Adam Kepecs**
Sponsor: Former URP Fund Fellowship
Acetylcholine and the basal forebrain in a sustained attention task.

Sarah Shareef, Harvard University
Advisor: **Dr. Christopher Vakoc**
Sponsor: Dorcus Cummings Scholar
Condensin localization along mitotic chromatin.

Ayse Trolander, Carleton College
Advisor: **Dr. Alea A. Mills**
Sponsor: Former URP Fund Fellowship
p63 point mutation causing EEC syndrome alters gene expression in vitro.

Anil Wadhwani, Northwestern University
Advisor: **Dr. Florin Albeanu**
Sponsor: Former URP Fund Fellowship
Neuromodulation of olfactory sensory input: A photon counting approach.

Unikora Yang, Brown University
Advisor: **Dr. Bo Li**
Fellowship: Steamboat Foundation Scholar
Establishing a novel attentional behavior test for mouse models of schizophrenia.

PARTNERS FOR THE FUTURE

PROGRAM DIRECTOR   **David Jackson**

PROGRAM ADMINISTRATOR   **Kathy LaForgia**

The Partners for the Future Program, established by Dr. James Watson in 1990, provides an opportunity for talented Long Island high school students to have hands-on experience in biomedical research at Cold Spring Harbor Laboratory. This highly competitive program is open to Long Island high school students in their junior year. Each high school science chairperson may nominate up to three students. The top candidates are interviewed by CSHL scientists and up to 11 top students are chosen to participate in the program. Students selected to the program are paired with a scientist mentor and spend a minimum of 10 hours per week, September through March of their senior year, conducting original research. At the conclusion, the students present their projects to an enthusiastic audience of the students' scientific mentors and colleagues, CSHL administrators, parents, and teachers. Although the students learn a great deal about modern biology and state-of-the-art research techniques, the main advantage of the program is that they are exposed to day-to-day life 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 2010–2011 Partners for the Future—chosen from among 24 semifinalist nominations—are listed below.

Partner	High School	CSHL Mentor	Laboratory
Laura Bergsten	Cold Spring Harbor High School	Adam Naguib	Lloyd Trotman
Gloria DiMino	Garden City High School	Mikala Egeblad	Mikala Egeblad
Rigina Gallagher	Sacred Heart Academy	Doreen Ware	Doreen Ware
Melissa Gluck	Sanford H. Calhoun High School	Hilary Cox	Partha Mitra
Daniel Kim	Syosset High School	Michael Wittinger	Alea Mills
Neha Kinariwalla	Sayville High School	Fred Van Ex	Robert Martienssen
Victoria Lellis	Harborfields High School	Guy Horev	Alea Mills
Kevin Liaw	The Wheatly School	Florin Albeanu	Florin Albeanu
Heather Szilagyi	Cold Spring Harbor High School	Peter Bommert	David Jackson
Jordan Woldenberg	Friends Academy	Sachin Ranade	Adam Kepecs
Deanna Zhu	Syosset High School	Zachary Lippman	Zachary Lippman

2010/2011 Partners for the Future participants

MEETINGS AND COURSES

ACADEMIC AFFAIRS

The meetings and courses program attracts scientists from all over the world to Cold Spring Harbor Laboratory to communicate, learn, and critique the latest ideas and approaches in the biological sciences. The program consists of advanced laboratory and lecture courses, as well as large meetings and biotechnology conferences that are held almost year-round. More than 8500 scientists ranging from graduate students and postdoctoral fellows to senior faculty came from around the world to attend these events at Cold Spring Harbor. A growing international program complements the main program of meetings and courses.

In 2010, the 30 laboratory and lecture courses that were held at the Laboratory attracted more than 1320 participants (including teaching faculty, students, and technicians). These courses covered a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of the courses remains to teach students the latest advances that can be immediately applied to their own research. Courses are always being evaluated and updated to include the latest concepts and approaches. Increasingly, many courses feature a strong computational component as biology grows ever more interdisciplinary, incorporating methodologies from computer science, physics, and mathematics.

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor. Their excellence and dedication make the course program work so well. We especially thank Drs. Ravi Allada, Vicki Alan, Beverly Errede, Ke Hu, Raymond Keller, John Kirby, Kristen Kroll, Amy Lee, John Murray, Larysa Pevny, Susan Lovett, Anca Segall, Beth Stevens, William Tyler, and Mei Zhen, who all retired this year after many years of service.

Grants from a variety of sources support the courses. The core support provided through the recently renewed grant from the Howard Hughes Medical Institute remains critical to our course program. The courses are further supported by multiple awards from the National Institutes of Health

Grace Patio—Symposium 75

(NIH) and the National Science Foundation (NSF), and additional support for individual courses is provided from various foundations and other sources. The courses also depend on equipment and reagents that are loaned or donated by a large number of companies. These are invaluable for making it possible to keep up with the latest technologies.

The Laboratory held 25 academic meetings this year, which brought together almost 7500 scientists from around the world to discuss their latest research. A major milestone for the meetings program was the 75th anniversary of the annual Symposium. To do justice to this anniversary, a special one-day celebration on "75 Years in Science—Biology, Society, and the Future" was organized by Jan Witkowski and held immediately prior to the Symposium. The celebration was also marked with a commemorative booklet with essays by leading scientists who have participated in these Symposia. The Symposium proper this year focused on the nucleus, reflecting the growing convergence between cell biological and mechanistic studies that are providing a major new synthesis in our understanding of how this command-and-control center of the cell operates. The Symposium attracted over 400 participants including notable nuclear biologists such as Günter Blobel, Joseph Gall, Robert Goldman, John Gurdon, Steven Henikoff, Robert Kingston, Barbara Meyer, Kim Nasmyth, Daniela Rhodes, and Pam Silver.

Cold Spring Harbor Laboratory meetings are unique in assembling a program with very few invited speakers. Instead, meeting organizers are encouraged to select talks from abstracts that are submitted on the basis of scientific merit, ensuring that the latest findings will be presented and that young scientists will have the chance to describe their work. The annual meetings on Gene Expression and Signaling in the Immune System, The Biology of Genomes, Retroviruses, and Translational Control were all oversubscribed, and many others attracted strong or record attendances. The Icelandic volcano disrupted attendance at spring meetings, but thankfully, travel restrictions were soon lifted. A new biotechnology conference on RNA and Oligonucleotide Therapeutics was held in April this year. Many of these meetings have become essential for those in the field and are held on a biannual basis. Partial support for individual meetings is provided by grants from NIH, NSF, foundations, and companies. Core support for the meetings program is provided by the Laboratory's Corporate Sponsor Program.

The joint Cold Spring Harbor/Wellcome Trust Conference series held at the genome campus south of Cambridge, England, included four meetings on Computational Cell Biology, Systems Biology: Networks, Infectious Disease Genomics, and Global Health, and Genome Informatics. Initiated in 2001, more than 3500 scientists have attended meetings on topics as diverse as genome informatics, host–pathogen interactions, and prion biology. The Wellcome Trust Conference team have now developed the tools and skills to manage the academic conference program independently.

Finally, 2010 saw the continued development of the Cold Spring Harbor Asia program, as a wholly owned affiliate of Cold Spring Harbor Laboratory with a mission to develop an educational hub based in Suzhou, China. Two inaugural Symposia—the Watson Symposium on Cancer and the Crick Symposium on Neuroscience—nine other scientific meetings, and one Summer School were held throughout the year.

The meetings and courses program staff is composed of a lean team of talented professionals, many who wear multiple "hats," who handle the complexities of database design, programming, web and multimedia design, and educational grants management.

<div align="right">

Terri Grodzicker
Dean
Academic Affairs

David Stewart
Executive Director
Meetings and Courses Program

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75TH COLD SPRING HARBOR LABORATORY SYMPOSIUM ON QUANTITATIVE BIOLOGY

Dynamic Organization of Nuclear Function

June 2–7	408 Participants
ARRANGED BY	**Terri Grodzicker, David Spector, David Stewart, and Bruce Stillman,** Cold Spring Harbor Laboratory

The decision to focus the historic 75th Symposium on the Dynamic Organization of Nuclear Function reflects the growing convergence between descriptive cell biological approaches to the nucleus and mechanism-driven approaches to nuclear processes that are now illuminating the structural and functional operations of the primary control center of the cell. Many previous Symposia have been devoted to nuclear organization and function, notably Genes and Chromosomes: Structure and Organization (1941); Genetic Mechanisms: Structure and Function (1956); Chromosome Structure and Function (1973); Chromatin (1977); Structures of DNA (1982); DNA and Chromosomes (1993); and Epigenetics (2004). Every decade has seen important advances in the field, so it is not surprising that past Symposia have shed light from many angles on the function of the central organelle of biology.

B. Stillman

A major theme of this year's Symposium addressed the emerging relationships between chromosome structure, genome function, and gene expression. Techniques have now emerged that allow the monitoring of chromatin states in cell lines and in vivo using molecular tags and sophisticated microscopy during differentiation. New methods to pinpoint the molecular mechanisms and intranuclear organization underlying transcriptionally active versus inactive chromatin are revealing extraordinary organizational complexity within the nucleus.

Epigenetic mechanisms such as DNA methylation and histone posttranslational modifications such as acetylation and methylation, imprinting, RNA interference, and gene silencing are also subject to dynamic organization in the nucleus. Epigenetic changes are important contributors to the pathogenesis of disease in humans, animals, and plants, and they may lie at the heart of many important gene–environment interactions. Research is illuminating how epigenetic mechanisms play important roles in regulating the structure and function of the genome, and the 2010 Symposium particularly highlighted the relationship between the genome, the epigenome, and the nuclear architecture and environment.

T. Grodzicker

The role of signaling in regulating transcription, mRNA export, and chromatin structure was also examined. For example, signaling from or at the nuclear envelope reveals novel mechanisms for influencing nuclear envelope morphology, breakdown, and cell differentiation. Chromatin dynamics was another major theme, both of the chromatin fiber and of chromatin-binding proteins involved in replication and repair, with new results discussed in areas such as the programming of DNA replication origins and their specificity. The latest developments in our understanding of mitotic and meiotic chromosome dynamics, as well as the roles for nuclear transport factors in cell cycle progression, were also covered.

Tremendous progress is also being made in analyzing the mechanisms of assembly for nuclear bodies and their functional roles. Regulated transport into and out of the nucleus through the nu-

M. Dundr, I. Raska, W. Bickmore, D. Spector

clear pore complex and the operation of selective transport mechanisms also featured prominently, leading to vigorous discussion of the competing models for facilitated transport, as well as reports of new factors required for mRNA export, tRNA shuttling, and pore complex assembly. There is growing evidence that pore permeability and nuclear leakiness change in a regulated way with cell differentiation and age.

The meeting addressed how nuclear organization and function are linked to the underpinnings of several pathophysiological states including the laminopathies such as A-type lamin dysfunction in the premature aging disease Progeria and the molecular determinants of cancer-prone symptoms of xeroderma pigmentosum and Cockayne syndrome, of the fatal bone marrow failure syndrome dyskeratosis congentia, and of the Roberts and Cornelia de Lange developmental syndromes. The relationship between nuclear architecture, aging, and disease was the subject of a masterful Dorcas Cummings Lecture on "The Unexpected Link between Premature Aging and Nuclear Organization" by Robert Goldman to a combined audience of scientists and lay friends and neighbors.

In arranging this Symposium, the organizers were dependent on the guidance of a broad cadre of advisors including Drs. Robert Goldman, Robert Kingston, Kim Nasmyth, Daniela Rhodes, and Pam Silver. Opening night speakers included John Gurdon, Rob Martienssen, Barbara Meyer, and Günter Blobel. Joseph Gall presented the Reginald Harris Lecture on "Transcription, RNA Processing, and Nuclear Bodies." Steven Henikoff closed the meeting with a masterful and thought-provoking summary.

This Symposium was attended by more than 400 scientists from over 25 countries, and the program included 68 invited presentations and 219 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 videoarchive, and a series of interviews with leading experts in the field was arranged during the Symposium and distributed as free video from the Cold Spring Harbor Symposium Chats website.

PROGRAM

Introduction
B. Stillman, *Cold Spring Harbor Laboratory*

Chromatin Modifications and Gene Expression
Chairperson: W. Bickmore, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

DNA Replication and Genome Integrity
K. Nasmyth, *University of Oxford, United Kingdom*

Reprogramming and Differentiation
Chairperson: T. de Lange, *The Rockefeller University, New York*

Reginald B. Harris Lecture: Transcription, RNA Processing, and Nuclear Bodies
J.G. Gall, *Carnegie Institution for Science, Baltimore, Maryland*

RNA Biology
Chairperson: D. Spector, *Cold Spring Harbor Laboratory*

Chromosome Structure and Mitosis
Chairperson: A. Amon, *Massachusetts Institute of Technology, Cambridge*

Heterochromatin Formation and Gene Silencing
Chairperson: G. Almouzni, *CNRS UMR218, Institut Curie, Paris, France*

Dorcas Cummings Lecture: The Unexpected Link between Premature Aging and Nuclear Organization
R. Goldman, *Northwestern University Medical School, Illinois*

Transcription Meets RNA Processing
Chairperson: J. Steitz, *HHMI/Yale University, New Haven, Connecticut*

Nuclear Periphery
Chairperson: W. Earnshaw, *University of Edinburgh, United Kingdom*

Chromatin Structure and Organization
Chairperson: S. Gasser, *Friedrich Miescher Institute for Biomedical Research, Basel, Switzerland*

Genome Regulation
Chairperson: E. Heard, *CNRS UMR128, Institut Curie, Paris, France*

Summary
Chairperson: Steven Henikoff, *Fred Hutchinson Cancer Research Center, Washington*

R. Tjian, C. Venter

T. Khoduchenko, J. Gall

S. Henikoff

MEETINGS

Neuronal Circuits

March 10–13   189 Participants

ARRANGED BY   **Cori Bargmann,** The Rockefeller University
Ed Callaway, Salk Institute for Biological Sciences
Dmitri Chklovskii, Janelia Farm Research Campus

In the quest to understand the brain, neuronal circuits represent a central level of description. Establishing connectivity in neuronal circuits seems as essential for solving the brain as having a geographic map for planning one's travel. Although such realization motivated Cajal to describe a variety of neuronal circuits using Golgi stains, his contributions were technologically limited. With the recent appearance of novel molecular genetic, imaging, and computational techniques, a comprehensive description of the wiring diagram—an old dream of neuroscientists—is about to become a reality.

Because technological advances have been made in different organisms and systems, we wanted to create a forum that brings together researchers working on different topics, yet focus on neuronal circuits. This meeting included six broad slide sessions covering the olfactory system, the visual system, methods, behavior learning and memory, motor systems, and the cortex, as well as a very interactive poster session.

For the third meeting of this kind, the response of the field was very enthusiastic. The meeting brought together participants from all over the world, most of whom made either oral or poster presentations. Invited talks were given by Larry Abbott (Columbia University), Richard Axel (Columbia University), Axel Borst (Max-Planck Institute of Neurobiology), Sreekanth Chalasani (Salk Institute), Karl Deisseroth (Stanford University), Marla Feller (University of California, Berkeley), Joseph Fetcho (Cornell University), Charles Gilbert (The Rockefeller University), Shaul Hestrin (Stanford University School of Medicine), Shawn Lockery (University of Oregon), Liqun Luo (Stanford University), Jeffrey Magee (LSUHSC), Ian Meinertzhagen (Janiela Farm Research Campus of HHMI), Markus Meister (Harvard University), May-

K. Briggman, S. Lockery

Britt Moster (CBM), Michael Nusbaum (University of Pennsylvania), I. Sebastian Seung (MIT), and Rachel Wilson (Harvard Medical School). The meeting provided an important clearinghouse for ideas and approaches and helped scientists in the field obtain the most up-to-date information, enabling them to meet, to network, and to establish collaborations. Based on the uniformly enthusiastic comments of the participants, the intensity of the oral and poster sessions, and the large crowds that stayed up late every night at the bar to discuss science, the meeting was a great success.

A highlight of the meeting program was the inaugural Larry Katz Memorial Lecture, made possible by the generous donation of many colleagues in the field. Excellent nominations were received. A committee composed of the three organizers and Liqun Luo selected one speaker: Christopher Harvey, Princeton University. He gave a wonderful talk about his graduate and postdoctoral work.

PROGRAM

Circuits and Behavior
Chairperson: L. Luo, *Stanford University, California*

Connectomics/Vision
Chairperson: D. Chklovskii, *HHMI Janiela Farm Research Campus, Ashburn, Virginia*

Cortex and Other Circuits
Chairperson: E.M. Callaway, *Salk Institute for Biological Studies, La Jolla, California*

Methods/Chemosensation
Chairperson: S. Lockery, *University of Oregon, Eugene*

Chemosensation/Motor
Chairperson: C. Bargmann, *The Rockefeller University, New York*

Neural Computations
Chairperson: M.-B. Moser, *Norwegian University of Science and Technology, Trondheim, Norway*

Larry Katz Memorial Lecture
C.D. Harvey, *Princeton University, New Jersey*

K. Kolkman, C. Root

Q. Wen, I. Rabinowich

E. Callaway, D. Chklovskii, C. Bargmann

PTEN Pathways and Targets

March 16–20   129 Participants

ARRANGED BY   **Suzanne Baker,** St. Jude Children's Research Hospital
Lewis Cantley, Beth Israel Deaconess Medical Center
Pier Paolo Pandolfi, Harvard Medical School
Ramon Parsons, Columbia University

This third meeting on PTEN pathways was enormously successful. The meeting brought together top scientists studying the PTEN/PI-3 kinase pathway using biochemical and molecular approaches as well as in vivo model systems. An exciting series of talks and poster presentations addressed the complex regulation of this signal transduction cascade and its critical role in normal development, cancer, aging and metabolism, autism, and other pathological disease states. The conference provided an opportunity for researchers of all levels to get together to discuss their latest research findings and technical approaches toward the analysis of the PTEN pathway. Particular emphasis was placed on pathway regulation and cross-talk, human cancer pathogenesis, disease models, and therapy.

D. Guerin, B. Manning, R. Shaw

A total of 76 investigators presented in the nine scientific sessions, with 41 platform sessions and 35 poster presentations and 129 registered attendees. The seven platform and two poster sessions were marked by dynamic and enthusiastic exchanges of new results. In the scientific sessions, many audience members participated in the question and answer sessions, and the poster sessions were well attended.

Essential funding for the meeting was provided by the National Cancer Institute, a branch of the National Institutes of Health.

PROGRAM

Upstream of PTEN
Chairperson: R. Parsons, *Columbia University, New York*

Downstream from PTEN
Chairperson: L. Trotman, *Cold Spring Harbor Laboratory*

PTEN Regulation
Chairperson: P. Devreotes, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Development and Disease Models
Chairperson: H. Wu, *University of California, Los Angeles*

mTORC1 and Intersecting Pathways
Chairperson: B. Manning, *Harvard School of Public Health, Boston, Massachusetts*

Therapy and Cancer Models
Chairperson: J. Engelman, *Massachusetts General Hospital, Boston*

Aging, Metabolism, and Cancer
Chairperson: A. Brunet, *Stanford University, California*

Systems Biology: Global Regulation of Gene Expression

March 23–27   284 Participants

ARRANGED BY   **Brad Bernstein**, Massachusetts General Hospital, Harvard Medical School, Broad Institute
Martha Bulyk, Brigham & Women's Hospital, Harvard Medical School
Harmen Bussemaker, Columbia University
Marian Walhout, University of Massachusetts Medical School

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 seventh conference in the series, like the ones in previous years, captured the continuing rapid progress and many new discoveries in this young and exciting field.

A total of 284 scientists attended the 4-day meeting, which featured 46 talks and 109 poster presentations covering a broad range of topics. Two keynote speeches were exciting highlights of the meeting. The first was presented by Dr. Kevin Struhl, who has been a leader in the area of transcriptional regulation for many years. Dr. Struhl presented a detailed story on the transcriptional and epigenetic changes that accompany cellular transformation in a model of breast cancer. The second keynote was presented by Dr. Steve Henikoff, a leader in the field of epigenetics with a long history of creatively integrating systems approaches into his research. Dr. Henikoff presented a global study of the role of histone variants and histone exchange in the regulation of the *Drosophila* genome.

M. Bulyk

H. Bussemaker

Compared to previous conferences, five aspects featured prominently in this year's meeting: (1) Next-generation sequencing technologies—representing increasingly standard means for data acquisition—are now being applied to study transcription factor localization, nucleosome positioning, chromatin modifications, and higher-order genome organization; the large amounts of new data have stimulated the generation of a plethora of new genomic methods, computational algorithms, resources, and biological insights; (2) new imaging technologies are allowing researchers to examine the dynamics of gene expression in vivo and in real time, and in some cases, at a single-molecule level; (3) new resources on the binding specificities of transcription factors and RNA-binding proteins continue to be created, providing opportunities for systematic analysis of gene regulatory networks in *Drosophila* and mammals; (4) there is much excitement around the newly appreciated and emerging roles for different classes of noncoding RNAs in genome regulation; and (5) new computational algorithms are being adopted to analyze the gene regulatory networks. Generally, the speakers in these sessions were young, with most being in the early stage of their careers. Finally, to help computational biologists and experiment scientists better communicate with each other, this year's meeting also included a premeeting workshop that was focused on introductory experimental and computational topics.

PROGRAM

Keynote Address: Mapping Genome-Wide Nucleosome Dynamics
S. Henikoff, *Fred Hutchinson Cancer Research Center, Seattle, Washington*

Keynote Address: An Epigenetic Switch That Links Inflammation to Cancer—Regulatory Circuits and Cancer Treatment
K. Struhl, *Harvard Medical School, Boston, Massachusetts*

Transcription
Chairperson: T. Hughes, *University of Toronto, Canada*

Transcriptional Regulatory Networks
Chairperson: X.S. Liu, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Chromatin
Chairperson: R. Morse, *Wadsworth Center, Albany, New York*

Epigenetics
Chairperson: J. Stamatoyannopoulos, *University of Washington, Seattle*

cis-Regulatory Logic
Chairperson: L. Mirny, *Massachusetts Institute of Technology, Cambridge*

Posttranscriptional Regulation
Chairperson: M. Walhout, *University of Massachusetts Medical School, Worcester*

Emerging Technology
Chairperson: B. Andrews, *University of Toronto, Canada*

Variation and Evolution
Chairperson: M. Bulyk, *Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts*

R. Hardison, G. Bernardi

L. Albert

A. Gerber, J. Lieb

RNA and Oligonucleotide Therapeutics

April 7–10   90 Participants

ARRANGED BY   **Ryszard Kole,** AVI BioPharma
Adrian Krainer, Cold Spring Harbor Laboratory
Bruce Sullenger, Duke University

This first conference on RNA and oligonucleotide therapeutics was focused on clinical trials and the 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" covered approaches ranging from antisense oligonucleotides and aptamers through modulation of splicing, targeting expanded triplet repeats, and immunostimulation to small interfering RNAs (siRNAs) and microRNAs (miRNAs) as potential drugs and targets. The all-important issue of RNA therapeutics delivery by a variety of methods was also covered. The applications of these varied technologies included rare diseases, cancer, cardiovascular diseases, and bacterial and viral infections.

A. Krainer, R. Kole, B. Sullenger

The participants came from 11 companies and from universities and research institutions from the United States and abroad. The seven scientific sessions featured 37 platform talks and 20 posters and included 90 registered attendees. Animated and insightful exchanges during the sessions continued throughout. A number of participants expressed interest in attending this conference next year. The meeting opened with a keynote 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 several companies including major funding from AVI Biopharma.

PROGRAM

Keynote Address: RNA and Therapeutic Modes
S. Altman, *Yale University, Connecticut*

Exon Skipping Therapy
Chairperson: R. Kole, *AVI BioPharma, Bothell, Washington*

siRNA, miRNA, and Aptamers as Therapeutics
Chairperson: B. Sullenger, *Duke University, Durham, North Carolina*

Posttranscriptional Control of Gene Expression
Chairperson: C. Thornton, *University of Rochester, New York*

Oligonucleotide Therapy
Chairperson: A. Krainer, *Cold Spring Harbor Laboratory*

Delivery of RNA Therapeutics
Chairperson: S. Dowdy, *HHMI/University of California, San Diego*

Oligonucleotide-Induced Immunostimulation and Other Mechanisms
Chairperson: S. Agrawal, *IDERA Pharmaceuticals, Cambridge, Massachusetts*

Gene Expression and Signaling in the Immune System

April 21–25   327 Participants

ARRANGED BY   **Doreen Cantrell,** University of Dundee, United Kingdom
Sankar Ghosh, Columbia University College of Physicians & Surgeons
Dan Littman, HHMI/New York University School of Medicine
Mark Schlissel, University of California, Berkeley

This meeting was held for the fifth time in 2010, taking place in the context of global travel restrictions due to the Icelandic volcano eruption. Although ~380 scientists registered for the meeting, 53 had to cancel due to flight cancellations in Europe. Nonetheless, ~25% of attendees were from outside of the United States, 41% were women, and 53% were either graduate students or postdocs. Although there are many immunology meetings, the unique aspect of this Cold Spring Harbor meeting is its focus on molecular and biochemical aspects of the development and function of the immune system. In addition, this meeting attracts a broad range of scientists who utilize the immune system as a model to ferret out basic principles of biological regulation.

M. Schlissel

Talks were presented by a mix of invited speakers and investigators selected from among a group of more than 200 submitted abstracts. Due to last-minute cancellations, several investigators were asked to substitute for scheduled speakers and they welcomed the opportunity. A significant number of women and junior investigators were asked to give talks. Most speakers focused almost exclusively on their unpublished work. A major new approach to the study of gene regulation permeated several talks— ChIP-Seq analysis of specific chromosomal protein DNA interactions. Meinrad Busslinger spoke about Pax5 and CTCF-binding sites in the IgHC locus that helped him define an important new potential regulatory element in the V region of that complex locus. David Schatz presented exciting new data on the genome wide binding of V(D)J recombinase components to specifically modified histones as well as RSS elements. Cornelius (Kees) Murre (University of California, San Diego) presented an update to his three-dimensional model of interphase structure of a complex genetic locus within nuclear space. The product of a collaboration with physicists and mathematicians, this model based on fluorescent in situ hybridization data helps explain how more than 100 immunoglobulin V_H gene segments distributed over greater than a megabase of DNA can recombine with D_H segments at roughly similar frequencies. Young investigator Greg Barton described the surprising discovery of how an enteric pathogen uses receptors from the innate immune system to trigger the expression of its own pathogenicity genes. Finally, in one of the last talks of the meeting, coorganizer Dan Littman revealed new details of how HIV interacts with but does not infect dendritic cells.

These talks highlighted the relevance of the data discussed at this meeting, not just to immunology but to the broader scientific community. Oral presentations were supplemented by two afternoon poster sessions that were extremely well-attended and provided a forum for all meeting participants (including many graduate students and postdocs) to share their most recent data. In addition, attendees took advantage of walking tours of the CSHL campus, a bountiful lobster banquet, and an evening performance by the Yale-based music group "The Cellmates," with dancing extending into the early morning hours.

Continuing support was obtained from the NIH/NIAID as well as from six corporate sponsors.
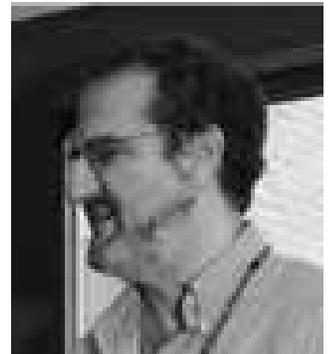
PROGRAM

Stem Cells and Early Developmental Decisions
Chairperson: S. Smale, *University of California School of Medicine, Los Angeles*

Regulation of Immune Cell Development
Chairperson: M. Schlissel, *University of California, Berkeley*

Chromatin Structure and Epigenetics
Chairperson: H. Singh, *HHMI/University of Chicago*

Antigen Receptor Gene Assembly and Modification
Chairperson: F. Alt, *HHMI/Children's Hospital, Boston, Massachusetts*

Signaling
Chairperson: A. Weiss, *HHMI/University of California, San Francisco*

Regulation of Immune Cell Function I
Chairperson: D. Littman, *HHMI/New York University School of Medicine*

Regulation of Immune Cell Function II
Chairperson: D. Cantrell, *University of Dundee, United Kingdom*

Innate Immunity
Chairperson: S. Ghosh, *Columbia University College of Physicians & Surgeons, New York*

S. Chatterjee, S. Volpi

G. Amarante-Mendes, T. Mak

E. Chan, M. Krangel, H. Singh

K. Murphy, T. Zhang

Vertebrate Organogenesis

Recent advances in stem cell biology are impressive and have strengthened our collective optimism that gene- and stem-cell-based therapies may eventually become realistic approaches in the treatment of many diseases, particularly those that have a significant pathological effect on organs and specific organ systems. Central to that goal is a clear understanding of the genes and mechanisms controlling vertebrate organogenesis in health and disease. Accordingly, the specific goals of this new conference was to bring together a diverse group of scientists working in various animal models and interested in studying different molecular, cellular, and genetic aspects of vertebrate organogenesis in basic and translational settings.

C. Blanpain, B. Hogan

Major topics (axis formation, nervous system, vascular system, epithelial organs, heart development, genito-urinary formation, endodermal organs, diabetes and cancer, and muscoskeletal formation) were chosen for detailed discussion, and these topics were all ultimately united by the use of approaches that pursue answers to key biological questions. Presentations on these topics provided

Poster session

a general overview of key questions, common problems, and progress achieved by investigators working in this field. The invited speakers were all leaders on each of the organs/tissues discussed. The organizers made selection of material from the submitted abstracts for short oral presentations. Two well-attended poster sessions were held during the conference.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health; the National Science Foundation; the Society for Developmental Biology; The Company of Biologists; Genentech; March of Dimes; and Novartis.

PROGRAM

Axis Formation
Chairperson: B. Hogan, *Duke University, Durham, North Carolina*

Development of the Nervous System
Chairperson: G. Oliver, *St. Jude Children's Research Hospital, Memphis, Tennessee*

The Vascular System in Health and Disease
Chairperson: L. Iruela-Arispe, *University of California, Los Angeles*

Epithelial Organ Morphogenesis
Chairperson: S. Pfaff, *The Salk Institute, La Jolla, California*

Developmental Mechanisms of Heart Development
Chairperson: J. Epstein, *University of Pennsylvania School of Medicine, Philadelphia*

Myopathies and Muscoskeletal Formation
Chairperson: G. Karsenty, *Columbia University, New York*

Developmental Origin of Genito-Urinary Defects
Chairperson: A. McMahon, *Harvard University, Cambridge, Massachusetts*

Endodermal-Derived Organs, Diabetes, and Cancer
Chairperson: K. Zaret, *University of Pennsylvania School of Medicine, Philadelphia*

B. Sosa-Pineda, L. Sussel, L. Iruela-Arispe

C. Miazga

Molecular Chaperones and Heat Shock Response

May 4–8   240 Participants

ARRANGED BY   **F. Ulrich Hartl,** Max-Planck Institute for Biochemistry
David Ron, New York University School of Medicine
Jonathan Weisssman, HHMI/University of California, San Francisco

This meeting continued the rich tradition of a forum where cutting-edge analysis of molecular mechanisms of protein folding is presented in the context of its biological importance and health relevance. The high attendance, large number of posters (94), and lively audience participation in the sessions and in the informal activities that followed are testament to the success of the meeting and to the successful balance of the sessions. The field of chaperone research is evolving and this meeting is surely evolving with it.

In addition to well-established biochemical and biophysical methods for studying chaperone function, this meeting featured presentations using single-molecule methods and advanced computational approaches to the study of signaling networks involved in control of chaperone gene expression. This year's meeting also featured two new sessions: one on Evolution and Regulation of Protein Folding Machines and the other on Manipulating Chaperone Networks and Protein Folding. The former provided a forum for work on the systems biology aspect of protein folding and the latter for work of special relevance to the prospects of translating the basic science to applications in health and biotechnology.

D. Kaganovich, B. Bukau

Among the cutting-edge themes explored were presentations of genetic interaction networks in mammalian systems. Talks covered protein trafficking in the cell that emphasized the fluidity of subcellular compartmentalization, intracellular protein turnover, connections between ribosomes and chaperones, and novel signaling pathways involved in protein-folding homeostasis.

This meeting was funded in part by the National Institute on Aging; the National Heart, Lung, and Blood Institute; the National Institute of General Medical Sciences; branches of the National Institutes of Health; and Enzo Life Sciences, Inc.

J. Bonner, D. Czyz, M. Hayer-Hartl

264 Meetings

PROGRAM

Chaperone Biochemistry and Protein Folding
Chairperson: J. Bardwell, *HHMI/University of Michigan, Ann Arbor*

Chaperone Function in Disease and Development
Chairperson: E. Deuerling, *University of Konstanz, Germany*

Evolution and Regulation of Protein-Folding Machines
Chairperson: J. Frydman, *Stanford University, California*

Manipulating Chaperone Networks and Protein-Folding Pathways

Chairperson: W. Balch, *The Scripps Research Institute, La Jolla, California*

Chaperones and Proteolysis
Chairperson: B. Sauer, *Massachusetts Institute of Technology, Cambridge*

Diseases of Protein Misfolding
Chairperson: A. Dillin, *The Salk Institute, La Jolla, California*

Quality Control and Protein Trafficking
Chairperson: R. Hegde, *University of California, San Francisco*

Y. Yanagitani

P. Slade, J. Kim

S. Quan

The Biology of Genomes

May 11–15   548 Participants

ARRANGED BY   **Susan Celniker,** Lawrence Berkeley National Laboratory
Andrew Clark, Cornell University
Chris Ponting, UK Medical Research Council, University of Oxford
George Weinstock, Washington University

This annual meeting marked the 23rd annual gathering of genome scientists at Cold Spring Harbor Laboratory. The meeting built upon the remarkable progress in the mapping, sequencing, annota-

tion, and analysis of genomes from many human individuals, "model organisms," other animals, and prokaryotes. New DNA-sequencing technologies have caused a steep rise in the quantity of sequences being generated, and the meeting reported many breakthroughs in our understanding of sequence variants that contribute to disease, including cancer and complex disease, and to phenotypic traits. The new data are also providing important insights into population structure for humans and other species. In addition to those on genome sequence, there were a number of reports of transcriptome and epigenomic profiling. Investigators from around the world attended the meeting, with more than 350 abstracts presented, describing a broad array of topics relating to the production and analysis of genomes from diverse organisms.

G. Weinstock, J. Watson

There were numerous reports on progress in using the new sequencing technologies, with mounting expectation for further advances as these continue to be applied widely. The keynote presentations were delivered by Cori Bargmann and Martin Blaser.

The ELSI (Ethical, Legal, and Social Implications) panel was chaired by Susan Wolf and the discussion topic was Returning Research Results to Participants in Large-Scale Genomics Studies.

Major sponsorship for this meeting was provided by Roche. Additional funding was provided by Illumina, Inc. and the National Human Genome Research Institute, a branch of the National Institutes of Health.

PROGRAM

Functional and Cancer Genomics
Chairpersons: L. Ding, *Washington University School of Medicine, St. Louis, Missouri;* P. Farnham, *University of California, Davis*

Genetics of Complex Traits
Chairpersons: D. MacArthur, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* C. Ober, *University of Chicago, Illinois*

High-Throughput Genomics and Genetics
Chairpersons: B. Graveley, *University of Connecticut Health Center, Farmington;* J. Shendure, *University of Washington, Seattle*

Computational Genomics
Chairpersons: L. Duret, CNRS, *Université Lyon 1, Villeurbanne, France;* J. Wortman, *University of Maryland School of Medicine, Baltimore*

ELSI Panel and Discussion: Returning Research Results to Participants in Large-Scale Genomics Studies
Moderator: S.M. Wolf, J.D., *University of Minnesota*
Panelists: Y. Joly, D.C.L., *McGill University, Canada;* K. Kato, Ph.D., *Kyoto University, Japan;* J. Kaye, Ph.D., *Oxford University, United Kingdom;* I. Kohane, M.D., Ph.D., *Harvard Medical School, Massachusetts*

Evolutionary Genomics
Chairpersons: L. Kruglyak, *Princeton University, New Jersey;* D. Petrov, *Stanford University, California*

Population Genomic Variation
Chairpersons: D. Adams, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* R. Nielsen, *University of California, Berkeley*

Keynote Speakers
C. Bargmann, *The Rockefeller University, New York*
Genetic Variation in Animal Behavior: Genes, Neurons, and Maybe Some Principles
M. Blaser, *New York University, New York*

Genetics and Genomics of Nonhuman Species
Chairpersons: K. Pollard, *Gladstone Institutes, San Francisco, California;* M. Purugganan, *New York University, New York*
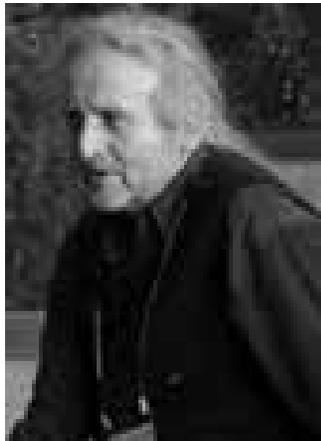
H. Blocker

D. Bentley, J. Mullikin

M. Banck, A. Beutler, P. Berylein

F. Casals, M. Zilversmit

The Cell Cycle

May 18–22   301 Participants

ARRANGED BY   **Sue Biggins,** Fred Hutchinson Cancer Research Center
Nicholas Dyson, Massachusetts General Hospital Cancer Center
Johannes Walter, Harvard Medical School

This biannual meeting is internationally recognized for its ability to bring together scientists who study cell cycle regulation in eukaryotes ranging from yeast to humans. As usual, the meeting began with a keynote talk from a leader in the field, and on this occasion, we were fortunate to have Dr.
Douglas Koshland, from the University of California, Berkeley, tell us about the initial discovery of cohesin and the subsequent contributions his lab has made to the field. After the keynote talk, the evening session continued with five talks focused on key findings about the regulation of cohesion.

S. Biggins, N. Dyson, J. Walter

The remainder of the meeting was organized around seven lecture sessions and two poster sessions, which focused on the major stages of the cell division cycle, with an emphasis in each session on the molecular mechanisms that govern cell cycle progression. Many of these sessions focused on long-standing problems in cell cycle control, including the transcriptional mechanisms controlling entry in the cell cycle, the mechanisms that initiate and control chromosome duplication, the mechanisms that trigger the complex events of chromosome segregation in mitosis, and the numerous checkpoint systems that ensure that cell cycle events occur in the correct order. Cutting-edge techniques to address problems as well as talks that emphasized modeling were represented. The meeting ended with a popular proteolysis session that brought together different perspectives from investigators who study the role of proteolysis in a variety of cell cycle processes. There were also a number of talks and posters about quiescence and other specialized states of the cell cycle.

The meeting also connected cell cycle regulation with cancer biology with reports about the functions of oncogenes and tumor suppressors in cell cycle control. As always, major model systems for cell cycle analysis were represented, and the striking phylogenetic conservation of cell cycle regulatory mechanisms was readily evident. The meeting also served to demonstrate the impact of whole-genome analysis on basic cell biology, and these achievements will no doubt continue to drive rapid progress in this field. It was another landmark meeting for the cell cycle field, and the participants continue to look forward to equally exciting meetings in future years.

PROGRAM

Keynote Address: Lessons Learned from Trying to Answer to a Higher Order
D. Koshland, *University of California, Berkeley*

Chromosome Cohesin and Condensation
Chairpersons: D. Morgan, *University of California, San Francisco;* J.-M. Peters, *Research Institute of Molecular Pathology, Vienna, Austria*

G1 and CDK Regulation
Chairpersons: S. Haase, *Duke University, Durham, North Carolina;* J. Lees, *Massachusetts Institute of Technology, Cambridge*

S Phase
Chairpersons: J. Diffley, *Cancer Research UK London Research Institute, United Kingdom;* B. Duronio, *University of North Carolina, Chapel Hill*

The DNA-Damage Response
Chairpersons: D. Toczyski, *University of California, San Francisco;* H. Piwnica-Worms, *HHMI/Washington University, St. Louis, Missouri*

Cell Cycle Regulatory Pathways
Chairpersons: P. O'Farrell, *University of California, San Francisco;* B. Edgar, *Universität Heidelberg, Germany*

Chromosome Segregation
Chairpersons: D. Pellman, *Dana-Farber Cancer Institute, Boston, Massachusetts;* T. Kapoor, *The Rockefeller University, New York*

Metaphase/Anaphase
Chairpersons: R. Medema, *University Medical Centre Utrecht, The Netherlands;* T. Davis, *University of Washington, Seattle*

Proteolysis
Chairpersons: B. Schulman, *St. Jude Children's Research Hospital, Memphis, Tennessee;* J. Pines, *University of Cambridge, United Kingdom*

B. Mardin, J. Keck

B. Gomez-Escoda

J. Witkowski, J. Watson

R. Medema

K. Tschop, K. Engeland

Retroviruses

May 24–29   500 Participants

ARRANGED BY   **Christopher Aiken,** Vanderbilt University
Jeremy Luban, University of Geneva

This was the 35th year of the Retroviruses Conference, and it coincided with the 35th anniversary of the Nobel Prize awarded to Renato Dulbecco, David Baltimore, and Howard Temin, "for their discoveries concerning the interaction between tumour viruses and the genetic material of the cell." Following tradition, the 13 sessions of the meeting (ten oral, three poster) focused on unpublished, basic research concerning mechanisms of retroviral replication and interactions with necessary host factors. HIV-1 took center stage, but there were presentations on other lentiviruses, HTLV-1, FIV, MLV, RSV, yeast viruses, and endogenous elements. A new area this year was XMRV, a new human retrovirus that has been associated with prostate cancer and chronic fatigue syndrome. The invited keynote speakers were Drs. Sandra Ruscetti and Ruslan Medzhitov. Dr. Ruscetti's talk laid the foundation for the large number of XMRV talks and posters that followed. Dr. Medzhitov described work on the autoimmunity that results from the accumulation of cDNA from endogenous retroviruses in the face of Trex1 deficiency and his work on novel mechanisms of intrinsic resistance to disease that permit animals to survive challenge with pathogens.

C. Aiken

Some highlights of the submitted research presentations were the demonstration that membrane microdomains have a role in virus capture and transfer by dendritic cells and that viral incorporation of a specific ganglioside (GM3) is critical for this process; advances in understanding the role of host factors TNPO3 and Nup358 in HIV infection, including the demonstration that such factors control HIV-1 integration targeting preference; identification of the first small-molecule inhibitor targeting the IN-LEDGF interaction; discovery and mechanism of novel capsid-targeting HIV inhibitors; identification of the host protein Mov10 as an inhibitor of retrovirus infection; demonstration of a positive role for the host factor ZASC1 in HIV Tat function; high-resolution X-ray structure of the HIV-1 CA pentamer; identification of host factors in-

J. Coffin, K. Muxiez-Forsyth, R. Temin, M. Kotler

P. Jolicoeur, F. Diaz-Griffero

volved in epigenetic silencing of retroviruses; identification of a role of Rab11a in HIV-1 Env incorporation; mechanistic insights into Vpu and Nef conteraction of the host restriction factor BST-2/Tetherin; and progress from several labs on the controversial role of XMRV in human disease.

Stephen Hare from the Cerepanov lab reported on their landmark achievement of the cocrystal structure of the a foamy virus integrase in complex with DNA, and Luban's group reported evidence that TRIM5 proteins can act as innate sensors for the retroviral capsid and participate in immune signaling, suggesting that TRIM5 may function as a pattern recognition receptor akin to TLR3 and RIG-I. Finally, Dan Littman presented exciting evidence that forcing HIV-1 to infected dendritic cells in culture induces a type I interferon response that depends on interactions of cyclophilin A with nascent Gag protein in the infected cell. Many other noteworthy advances were reported but cannot be listed here due to space constraints.

On the final evening of the meeting, a special session celebrated the 35th anniversary of the Nobel Prize awarded to the codiscoverers of reverse transcriptase, Dr. Howard Temin and Dr. David Baltimore. Two invited speakers for the occasion, Dr. Rayla Temin and Dr. Baltimore, were introduced by Dr. John Coffin and Dr. Stephen Goff, respectively. The legacies of Temin and Baltimore were strongly evident during the session, not only from the historical descriptions of their monumental achievements, but also owing to the fact that the majority of meeting attendees confirmed that they received scientific training, either directly or indirectly, from one of these giants of the field. Dr. James Watson was present to celebrate his fellow Nobel laureates.

PROGRAM

Entry
Chairpersons: J. Mak, *The Burnet Institute, Melbourne, Australia;* C. Berlioz-Torrent, *INSERM U1016, Paris, France*

RT and Trim5
Chairpersons: F. Diaz-Griffero, *Albert Einstein College of Medicine, Bronx, New York;* W. Johnson, *Harvard Medical School, Boston, Massachusetts*

Goin' Nuclear
Chairpersons: M. Naghavi, *University College Dublin, Ireland;* E. Bacharach, *Tel Aviv University, Israel*

Keynote Address
S. Ruscetti, *National Cancer Institute, Frederick, Maryland*
Understanding the Biological Effects of the Human Retrovirus XMRV: Lessons Learned from Studying Murine Retroviruses

H. Taylor, O. Ciagoz

S. Garforth, C. Lwatula

Apobec

Chairpersons: V. Simon, *Mount Sinai School of Medicine, New York;* Y.-H. Zheng, *Michigan State University, East Lansing*

Integration

Chairpersons: M. Roth, *UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey;* P. Cherepanov, *Imperial College London, United Kingdom*

RNA

Chairpersons: J.-C. Paillart, *CNRS-Université de Strasbourg, France;* W.-S. Hu, *National Cancer Institute, Frederick, Maryland*

Assembly

Chairpersons: F. Bouamr, NIAID, *National Institutes of Health, Bethesda, Maryland;* A. Ono, *University of Michigan Medical School, Ann Arbor*

Keynote Address

R. Medzhitov, *HHMI/Yale University, Connecticut*
Understanding the Biological Effects of the Human Retrovirus XMRV: Lessons Learned from Studying Murine Retroviruses

Tetherin and XMRV

Chairpersons: S. Neil, *King's College London, United Kingdom:* I. Singh, *University of Utah, Salt Lake City*

Structure, Genome, and Defense

Chairpersons: F. Bushman, *University of Pennsylvania, Philadelphia;* D. Unutmaz, *New York University School of Medicine*

Special Celebration

35th anniversary of the Nobel Prize in Physiology or Medicine, "for their discoveries concerning the interaction between tumor viruses and the genetic material of the cell"

Introduction by John Coffin

R. Temin, *University of Wisconsin, Madison*

Introduction by Stephen Goff

D. Baltimore, *California Institute of Technology*

Tetherin and Pathogenesis

Chairpersons: D. Evans, *Harvard Medical School, Boston, Massachusetts;* M. Pizzato, *University of Geneva, Switzerland*

T. Izumi, K. Sato

T. Grodzicker, D. Littman

J. Komano, E. Urano

75 Years in Science: Biology, Society, and the Future

June 1   212 Participants

ARRANGED BY   **Jan Witkowski,** Banbury Center, Cold Spring Harbor Laboratory

Reginald Harris became the director of the Biological Laboratory at Cold Spring Harbor in 1924. He set about revitalizing the Laboratory, making biophysics the new focus of research and, most notably, established the Cold Spring Harbor Laboratory Symposia on Quantitative Biology. Their goal, Harris wrote, was "to consider a given biological problem from its chemical, physical and mathematical, as well as from its biological aspects."

The first symposium was held in 1933 and they have been held annually ever since, with the exception of 1943–1945, surely making them one of the longest-lived series of meetings in the biological sciences. The success of the Symposia was due not only to the quality of the science presentations, but also to the very generous funding by two foundations, the Rockefeller Foundation and the Carnegie Foundation. These together helped support the meetings from 1934 through 1960.

The Symposia topics have always been at the forefront of contemporary research. Prior to World War II, biophysics research was the theme of the Symposia after the war; genetics came to dominate the Symposia for more than 20 years, culminating in the 1996 Symposium, The Genetic Code. Since then, Symposia topics have ranged over all aspects of experimental biology—DNA, RNA, and proteins; cell biology; developmental biology; human genetics; cancer; neuroscience; physiology; and immunology and virology.

Such a storied history had to be celebrated and so a 1-day meeting was held immediately before the start of the 75th Symposium. We included two examples of the power of molecular genetics when applied to human beings—what is being revealed of our origins and the use of DNA fingerprinting to free the wrongly convicted. Molecular genetics was also the foundation of two presentations on key areas of biomedical research: cancer and neurodevelopmental disorders. Finally, we looked deep into the crystal ball with a talk on synthetic genomes. The banquet, always a lively event, was further enlivened by Sydney Brenner who was the after-dinner speaker, and he and Jim Watson cut a cake celebrating the occasion.

S. Henikoff, J. Gall

Reginald Harris left a legacy that has stimulated the generations of biologists who have come each year to talk, argue, and gossip at the Symposium. Just as Harris promoted biophysics as the key to a deep understanding of the nature of living organisms, so contemporary organizers promote molecular approaches for the investigation of biological systems. As we celebrated the occasion of the 75th Symposium, there was every reason to believe that the Symposia will continue to fulfill Harris's vision.

PROGRAM

Opening Remarks
B. Stillman, *Cold Spring Harbor Laboratory*
Chairperson: R. Tjian, *Howard Hughes Medical Institute, Chevy Chase*

Devising Cancer Therapies
Chairperson: C. Sawyers, *Memorial Sloan-Kettering Cancer Center, New York*

Commentary
T. Jacks, *Massachusetts Institute of Technology, Cambridge*

The Journey of Man: A Genetic Odyssey
S. Wells, *National Geographic Society, Washington, District of Columbia*

Commentary
H.L. Gates, Jr., *Harvard University, Cambridge, Massachusetts*

Fulfilling the Promise of Molecular Medicine in a Developmental Brain Disorder
M. Bear, *Massachusetts Institute of Technology, Cambridge*

Commentary
S. Landis, *National Institute of Neurological Disorders & Stroke, Bethesda, Maryland*

The Wrongly Convicted: DNA and Justice
P. Neufeld, *Innocence Project, New York*

Commentary
J.C. Venter, *J. Craig Venter Institute, Rockville, Maryland;* R. Roberts, *New England BioLabs, Inc., Ipswich, Massachusetts*

Closing Remarks
J.D. Watson, *Cold Spring Harbor Laboratory*

E. Heard, J. Haber

Glia In Health and Disease

July 22–26   193 Participants

ARRANGED BY   **Beth Stevens,** Children's Hospital/Harvard Medical School
William Talbot, Stanford University

Glial cells constitute 90% of cells in the human nervous system, but their roles in the developing, adult, and diseased brain are still poorly understood. In this summer's meeting, students and scientists from across the world gathered to discuss their recent progress. In the first part of the meeting, sessions focused on understanding the normal roles of glia, including sessions on the genetic analysis of glial function, neural development, myelination, axon–glial interactions, microglia function, and astrocyte function at synapses and blood vessels. In the last half of the meeting, sessions focused on recent progress on understanding the roles of glia in CNS regenerative failure and disease processes, including glioma, epilepsy, and multiple sclerosis. There was active discussion related to the protective and destructive roles of "reactive gliosis" in CNS injury and neurodegenerative diseases including ALS (Lou Gehrig's disease) and glaucoma.

W. Talbot, B. Stevens

One of the highlights of the meeting was an animated roundtable discussion led by Ben Barres and Martin Raff on key issues related to glial biology and life in science. Poster sessions were well attended with active discussion. There was again a sense of excitement that our understanding of the function of glia is quickly moving forward, with glia having central roles in normal brain development, function, and disease. This excitement was reflected in the presence of several top journal editors interested in enhancing their coverage of latest advances concerning glia.

This meeting was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.

L. Goutebroze, J. Ness-Myers

A. Agarwal

A. Giangrande, M. Raff

PROGRAM

Genetic Analysis of Glial Function
Chairpersons: B. Popko, *University of Chicago, Illinois;* M. Freeman, *University of Massachusetts Medical School, Worcester*

Myelinating Cells
Chairpersons: K. Jessen, *University College London, United Kingdom;* P. Brophy, *University of Edinburgh, United Kingdom*

Axon–Glial Interactions
Chairpersons: J. Chan, *University of California, San Francisco;* E. Peles, *Weizmann Institute of Science, Rehovot, Israel*

Glial Function at Synapses
Chairpersons: D. Bergles, *Johns Hopkins University, Baltimore, Maryland;* G. Corfas, *Children's Hospital, Boston, Massachusetts*

Microglia Function in Health and Disease
Chairpersons: A. Bessis, *Ecole Normale Supérieure, Paris, France;* M. Carson, *University of California, Riverside*

Roundtable Discussion: Glia in Health and Disease—Open Questions and Future Directions
Chairpersons: B. Barres, *Stanford University School of Medicine, California;* M. Raff, *University College London, United Kingdom*

Gliovascular Interactions and Astrocyte Function
Chairpersons: R. Daneman, *University of California, San Francisco;* M. Nedegaard, *University of Rochester, New York*

CNS Injury and Disease
Chairpersons: M. Sofroniew, *University of California School of Medicine, Los Angeles;* H. Zong, *University of Oregon, Eugene*

Glial Development and Epigenetic Mechanisms
Chairpersons: D. Rowitch, *University of California, San Francisco;* P. Casaccia, *Mount Sinai School of Medicine, New York*

H. Colognato, D. Parkinson

M.-L. Schmiedt

Mechanisms and Models of Cancer

August 17–21   436 Participants

ARRANGED BY   **Dafna Bar-Sagi,** New York University Medical Center
Jacqueline Lees, MIT Center for Cancer Research
Charles J. Sherr, HHMI/St. Jude Children's Research Hospital
William Weiss, University of California, San Francisco

Molecular alterations affecting tumor suppressor genes and oncogenes have long been recognized to spark the development of human cancers. During the last few years, steady advances in technological developments have allowed a more exquisitely detailed dissection of signaling networks that are per-turbed during different stages of tumor initiation and progression and that determine the responses of cancers to both conventional and targeted therapeutics. In addition, there has been the emerging appreciation that interactions of tumor cells with their tissue microenvironment conjoin to alter tumor cell metabolism, affect the manner by which a variety of host inflammatory and immune responses affect cancer progression, and determine properties of invasion and metastasis.

W. Weiss, J. Lees

This third meeting convened an international group of investigators whose collective work focused on these themes. Oral and poster presentations provided numerous examples of how new insights have been gained from application of interdisciplinary approaches that utilize genetics, developmental biology, genomics and proteomics, and model organisms (principally sophisticated mouse models) to advance the development of rational therapeutics. A keynote address by Eileen White illustrated the importance of autophagy (self-cannibalization) as a pathway to which cancer cells show addiction and demonstrated its relevance for therapeutic targeting. There were eight oral sessions and two poster sessions. The meeting will be held again in the summer of 2012.

This conference was funded in part by the National Cancer Institute, a branch of the National Institutes of Health.

PROGRAM

Apoptosis and Autophagy
Chairperson: E. White, *Rutgers University, New Brunswick, New Jersey*

Keynote Address
J. Brugge, *Harvard Medical School, Massachusetts*

Signaling Mechanisms I
Chairperson: R. Shaw, *The Salk Institute for Biological Studies, La Jolla, California*

Mouse Models
Chairperson: C. Abate-Shen, *Columbia University Medical School, New York*

Rb and p53 Networks
Chairperson: K. Vousden, *Beatson Institute for Cancer Research, Glasgow, United Kingdom*

Microenvironment, Inflammation, and Metastases
Chairperson: L. Parada, *University of Texas Southwestern Medical Center, Dallas*

Experimental Therapeutics
Chairperson: R. DePinho, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Genomics, Proteomics, Epigenetics
Chairperson: F. White, *Massachusetts Institute of Technology, Cambridge*

Signaling Mechanisms II
Chairperson: M. Eilers, *University of Würzburg, Germany*

Molecular Genetics of Bacteria and Phages

August 24–28   169 Participants

ARRANGED BY   **James Hu,** Texas A&M University
Petra Levin, Washington University
Malcolm Winkler, Indiana University Bloomington

This meeting featured 74 oral presentations and 82 poster presentations. Bacteria and phages continue to provide critical insights into diverse areas of biology, ranging from ecology, evolution, and diversity, to gene expression and development, to infectious diseases. Exciting presentations covered all of these topics and continued the historical strength of Phage meetings in combining long-term themes of molecular microbiology with cutting-edge approaches and exciting new research areas. A highlight of the meeting was the keynote lecture by long-time participant Susan Gottesman, who coupled a review of her career in bacterial molecular biology with recognition of the 100th anniversary of the birth of Jacques Monod. Another celebration was for the long, continuous participation of Wisconsin's Waclaw Szybalski, whose generous support of the CSHL library was warmly recognized.

J. Hu, P. Levin

The opening session focused on development, cell signaling, and cell–cell interactions. Session chair Mike Laub touched on several recurring themes of the meeting in his description of how *Caulobacter* uses the kinetics of phosphorylation and dephosphorylation of key regulators to control its asymmetric cell division. Michelle Giglio presented an overview of the Human Microbiome Project and chaired the second session with many other thought-provoking examples of how genomes and metagenomes are opening new areas of biology. Ry Young chaired the session on Bacterial Cell Surfaces, and presented a talk on the discovery of spanin proteins that mediate the destruction of the inner membrane, peptidoglycan, and outer membrane by phages of Gram-negative bacteria.

Gail Christie spoke on the functions and evolutions of superantigen-encoding pathogenicity islands that are mobilized by helper phages. Bryce Nichols' talk concerned the detection of nanoRNAs that can prime transcription initiation in vivo. Alan Wolfe spoke on the role of pathways that mediate the synthesis of acetyl phosphate and acetylation of target proteins. Robert Britton started his session with a talk on the role of conserved GTPases in ribosome assembly. David Rudner, who chaired the last session, talked about new proteins that regulate DNA replication during sporulation. Regular talks and posters were presented by a mix of PIs, postdocs, and advanced graduate students, whose high-quality presentations are a unique aspect of this meeting.

PROGRAM

Development, Cell Signaling, and Cell–Cell Interactions
Chairperson: M. Laub, *HHMI/Massachusetts Institute of Technology, Cambridge*

Molecular Evolution, Systems Biology, and Genomics
Chairperson: M. Giglio, *University of Maryland School of Medicine, Baltimore*

Bacterial Cell Surfaces
Chairperson: R. Young, *Texas A&M University, College Station*

Bacteriophage Biology
Chairperson: G. Christie, *Virginia Commonwealth University, Richmond*

Mechanism and Regulation of Transcription
Chairperson: B. Nickels, *Rutgers University, Piscataway, New Jersey*

Bacterial Metabolism and Physiology
Chairperson: A. Wolfe, *Loyola University, Chicago, Illinois*

Translation and Posttranscriptional Regulation
Chairperson: R. Britton, *Michigan State University, East Lansing*

Keynote Address: Beyond Transcription: Complex Regulatory Networks for Responding to Changing Environments
S. Gottesman, *National Cancer Institute*

DNA Replication, Recombination, and Transposition
Chairperson: D. Rudner, *Harvard Medical School, Boston, Massachusetts*

K. Potrykus, M. Cashel

A. Labner-Oleson, D. Chattoraj

F. Pouillot, H. Blois

Nuclear Receptors and Disease

Aug. 31–Sept. 4   205 Participants

ARRANGED BY   **Keith Yamamoto,** University of California, San Francisco
Ron Evans, Salk Institute Biological Studies
Jerrold M. Olefsky, University of California, San Diego

Forty-eight nuclear receptors are encoded in the human genome, and, collectively, they exert broad control over most biological processes. The theme of this meeting was to bring together scientists from different disciplines to exchange ideas and forge new insights into the biology of nuclear receptors. Accordingly, the meeting focused on the role of nuclear receptors in metabolism and cancer. This represented the third time the nuclear receptor community gathered at the Cold Spring Harbor Laboratory for their biannual meeting.

J. Olefsky, R. Evans, K. Yamamoto

The meeting brought together an enthusiastic group of participants engendering lively discussions and debates, and an open exchange of data and ideas. The invited Keynote talks were given by David Mangelsdorf, Bruce Spiegelman, and Laurie Goodyear. Other invited speakers included Johan Auwerx, Juleen Zierath, Walter Wahli, Katja Lamia (The Salk Institute for Biological Studies), Alan Saltiel (University of Michigan), DaYoung Oh (University of California, San Diego), Philipp Scherer (University of Texas Southwestern Medical Center), Michael Stallcup (University of Southern California), Susanne Mandrup (University of Southern Denmark), Bert O'Malley (Baylor College of Medicine), Donald McDonnell (Duke University Medical Center), Myles Brown (Dana-Farber Cancer Institute), Vincent Giguere (McGill University), Mitchell Lazar (University of Pennsylvania School of Medicine), Christopher Newgard (Duke University Medical Center), Christopher Glass (University of California, San Diego), Gokhan Hotamisligil (Harvard School of Public Health), Laszlo Nagy (University of Debrecen), W. Lee Kraus (University of Texas Southwestern Medical Center), Peter Tontonoz (University of California, Los Angeles), and Gordon Hager (NCI, National Institutes of Health).

PROGRAM

Keynote Addresses
D.J. Mangelsdorf, *University of Texas Southwestern Medical School, Dallas*
Nuclear Receptor Modulators (NuRMs) in Worms
B. Spiegelman, *Dana-Farber Cancer Institute, Boston, Massachusetts*
PPARγ and the Antidiabetic PPARγ Ligands: A New Look at an Old Friend
L. Goodyear, J*oslin Diabetes Center, Boston, Massachusetts*
Skeletal Muscle Metabolism: AMPK and Beyond

Physiology and Metabolism
Chairperson: A. Saltiel, *University of Michigan, Ann Arbor*

Inflammation and Metabolic Disease
Chairperson: B. Desvergne, *University of Lausanne, Switzerland*

Ligands and Cofactors
Chairperson: R.M. Evans, *The Salk Institute, La Jolla, California*

Cancer
Chairperson: C. Glass, *University of California, San Diego*

Mechanisms of Insulin Resistance and Adipogenesis
Chairperson: J.M. Olefsky, *University of California, San Diego*

Inflammation II
Chairperson: S. Mandrup, *University of Southern Denmark, Odense*

Chromatin and Transcription
Chairperson: M. Stallcup, *University of Southern California, Los Angeles*

K. Duszka, N. Herman-Kleiter

J. Judkins, D. Mangelsdorf

M. Rozendaal, J. Nwachukwu

D. Oh, E. Bae, K. Kemper

Personal Genomes

This special meeting on personal genomes acknowledges the increasing number of personal genomes that are being sequenced in a clinical context, for identifying disease-causing mutations and, in some cases, for diagnosis and treatment. As the availability and use of next-generation, and soon third-generation, sequencing instruments become more widespread, the critical questions that impact our understanding of human biology and disease are more likely to be addressed by large and small laboratories.

The meeting provided an opportunity to explore a number of important themes (see below). It featured a combination of talks and posters and included a keynote talk and ethics panel discussion that addressed the societal and medical impacts of the new sequencing technologies and their applications. The critical success and timeliness of the meeting can be judged from the considerable enthusiasm of the audience

G. Church

and the increased number of attendees from clinical disciplines, including several plenary speakers. The collective decision was taken to hold the meeting for a third time at Cold Spring Harbor in the fall of 2011.

L. White, G. Scott, A. Shah

J. Kwan, S. Calvo

PROGRAM

Personal Genome Landscape
Chairpersons: D. Conrad, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom;* J. Wang, *Beijing Genomics Institute, Shenzhen, China*

Keynote Addresses
E.D. Green, *National Human Genome Research Institute, Maryland*
Genomics in 2K10 and Beyond: Charting a Course for Genomic Medicine
L.E. Hood, *Institute for Systems Biology, Seattle, Washington*
Systems Genetics and Systems Biology
H. Greely, *Stanford University, Stanford, California*
Preparing for the Coming Tsunami of Clinical Genomic Information

Ethics Panel: Teaching Genomics and Related Ethics to Medical Professonals

Personal Cancer Genomes
Chairpersons: S. Grimmond, *University of Queensland, St. Lucia, Australia;* S. Jones, *BC Cancer Agency, Vancouver, Canada*

Personal Transcriptomes and Other Applications
Chairpersons: P. Laird, *University of Southern California, Los Angeles;* R. Myers, *HudsonAlpha Institute for Biotechnology, Huntsville, Alabama*

Inherited Diseases
Chairpersons: L. Jorde, *University of Utah School of Medicine, Salt Lake City;* D. Altshuler, *Broad Institute, Cambridge, Massachusetts*

Technologies for Personal Genomes
Chairperson: G. Church, *Harvard Medical School, Boston, Massachusetts*

A. Dopazo, L. Brizuela

E. Green, H. Sussman

Translational Control

This meeting attracted participants from around the world, and included Jamie Cate, Rachel Green, Eric Klann, and Paul Schimmel as keynote speakers, eight platform sessions and three poster sessions that covered 337 abstracts. Novel findings on the mechanism of translation included the first high-resolution crystal structure of the eukaryotic 80S ribosome, a 5.5 Å cryo-EM structure of the wheat ribosome, and multiparticle cryo-EM analysis of translocating ribosomes that revealed new intermediate tRNA hybrid states.

C. Hellen, A. Hinnebusch

There were several breakthroughs regarding the mechanism of initiation. Interaction of the eIF4G subunit of the mRNA cap-binding complex with poly(A)-tail binding protein PABP was found to be dispensable for initiation in yeast, being functionally redundant with an RNA-binding domain in eIF4G. Among many other findings, evidence was presented that ribosomes can circumvent proteins or oligonucleotides bound to the 5′UTR, presumably by pathways independent of linear scanning. Important developments concerning elongation and termination included new findings on yeast No-Go mRNA decay quality control. Several lines of evidence suggest that the termination and ribosome recycling machineries are more closely related than previously anticipated. New methods were described for monitoring the dynamics of translation elongation in living cells, and ribosomal profiling by deep-sequencing of ribosome-protected mRNA fragments. Pharmacological approaches to suppressing nonsense mutations were described as a new

Wine and cheese party

therapeutic approach to treating genetic diseases.

In the arena of translational control in disease, new results show that a lack of rRNA modification impairs cell cycle arrest in early oncogenesis by preventing a switch to IRES-mediated translation of tumor suppressor p53. New insights into virus–host interactions were obtained, and novel initiation mechanisms were reported for plant positive-strand RNA viruses. In calicivirus translational reinitiation, ribosomes can reach start sites not only by direct positioning, but also by movement along the viral RNA in a prokaryotic-like fashion. Regarding translational control in development and the CNS, a novel requirement for the eIF2 kinase PERK in regulating LTD was demonstrated, which has important behavioral consequences in un-learning previous associations. In *Aplysia* sensory neurons, the effects of TOR in increasing cap-dependent translation were shown to be independent of the major eIF4E- binding protein and S6 kinase. TORC2 was found to associate with polyribosomes and cotranslationally phosphorylate AKT, regulating its stability.

A. Contreras, A. Rodrigues, E. Bueno

Finally, new insights into connections between translation and mRNA turnover were also reported, including the fact that exon junction complexes (EJCs) are not deposited at all exon–exon junctions. There is evidence that EJCs associate with the nonsense-mediated mRNA decay (NMD) factors SMG1 and UPF1 in a way that is promoted by the cap-binding protein of the pioneer translation initiation complex and, subsequently, SMG6 by a mechanism that is inhibited by EJC association with the UPF3 NMD factor. This represents just a sampling of the many new findings presented at the meeting, with vigorous discussion by graduate students, postdocs, and professors at both oral sessions and poster sessions. The informality of the environment at Cold Spring Harbor encourages intense and fruitful debate and discussion of novel ideas and approaches.

PROGRAM

Ribosome Structure and Function
Chairperson: J. Dinman, *University of Maryland, College Park*

Factors and Mechanisms
Chairperson: J. Lorsch, *Stanford University, California*

Viruses and Disease
Chairperson: G. Meyers, *Bundesforschungsanstalt für Viruskrankheiten der Tiere, Tübingen, Germany*

mRNA Turnover
Chairperson: L. Maquat, *University of Rochester Medical Center, New York*

Development, CNS, and Signaling
Chairperson: W. Sossin, *McGill University, Montréal, Canada*

***Trans*-Acting Regulators**
Chairperson: P. Fox, *The Cleveland Clinic, Ohio*

Elongation and Termination
Chairperson: D. Bedwell, *University of Alabama, Birmingham*

RNA Regulatory Elements
Chairperson: S. Thompson, *University of Alabama, Birmingham*

Axon Guidance, Synaptic Plasticity, and Regeneration

September 21–25   364 Participants

ARRANGED BY   **Graeme Davis,** University of California, San Francisco
Alex Kolodkin, Johns Hopkins University
Carol Mason, Columbia University

The human brain has billions of nerve cells (neurons), and each neuron is typically connected to hundreds of other neurons via synapses in a highly precise fashion. This complex neural wiring underlies the ability of humans, and other animals, to interact with the outside world to learn and to perform complex behaviors. Defects in the development of neural connections are being increasingly linked to the cause of neurological disease. One of the major challenges for the field of neuroscience is to understand how nerve connections are made accurately and reliably. In the past decade, our understanding of the mechanisms that control axon growth and guidance, synaptogenesis, and the remodeling of neural circuits during development has progressed rapidly from phenomenology to the identification of specific molecular control mechanisms.

S. Arber, G. Davis

This seventh meeting in the series focused on key issues in axon guidance, circuit formation, synaptogenesis, and plasticity. For the first time, it highlighted regeneration and included sessions devoted to particular problems in the assembly, plasticity, and repair of the nervous system. Speakers were chosen from among the participants submitting abstracts by session chairs who are leaders in the field. The response of the field to the 2010 conference was one of overwhelming enthusiasm, with more than 360 participants, 257 of whom submitted abstracts; 49 abstracts were selected for talks, in seven sessions, the remaining abstracts being presented as posters. Senior researchers, starting assistant professors, postdoctoral fellows, and graduate students were well represented as speakers and participants. Session chairs at the meeting were well-balanced between men and women and had a clear international presence with participants from Europe and Asia.

Many of the outstanding talks this year were by graduate students. All of the major areas of research in the field were covered, as were all of the major approaches (cellular, physiological, anatomical, molecular, dynamic imaging, biochemical, and genetic). In addition, there were three keynote addresses. The first, by a speaker outside of the field of neural development, was given by Professor Peter Devreotes and reviewed his work on the motility, polarization, and directional sensing during chemotaxis of the single cell forms of *Dictyostelium*. The second and third keynote lectures were a pairing of speakers who gave provocative talks on how specificity is achieved and the limits to exact circuit formation and function. Tom Jessell focused on work that extends his findings regarding transcriptional regulation of cell identity to the afferent–target interactions leading to motor output in the vertebrate spinal cord. Eve Marder spoke on the physiological and computational aspects of compensation in network performance, with the lobster CNS as her model.

The meeting provided an important forum for ideas and approaches and helped scientists in the field get the most up-to-date information, enabling them to meet, network, and establish collaborations. As at the previous meeting, a need was identified for an additional poster session because the number and quality of abstracts submitted for posters was so high. The three poster sessions were extremely well attended. In addition, fewer talks were scheduled in the evening, and a talk ses-

sion after the evening keynote was eliminated to allow time for discussion and attendee interactions. Based on the enthusiastic comments of the participants, the intensity of the oral and poster sessions, and the large crowds that stayed up late every night at the bar to discuss science, the meeting was a great success.

This meeting was funded in part by National Institute for Neurological Diseases and Stroke, a branch of the National Institutes of Health.

PROGRAM

Axon to Synapse I
Chairpersons: K. Martin, *University of California, Los Angeles;* Y. Zou, *University of California, San Diego*

Synapse to Circuit I
Chairpersons: M. Feller, *University of California, Berkeley;* A. Chedotal, *Institut de la Vision, INSERM, Paris, France*

Special Lecture: The Cell's Compass: Chemoattractant Bias of an Excitable Network of Parallel Signaling Pathways Directs Cell Migration
P. Devreotes, *Johns Hopkins University School of Medicine, Maryland*

Stem Cells, Regeneration, and Disease I
Chairpersons: Y. Jin, *HHMI/University of California, San Diego;* F. Bradke, *Max-Planck Institute of Neurobiology, Martinsried, Germany*

Axon to Synapse II
Chairpersons: S. Arber, *Biozentrum, Basel, Switzerland;* B. Sabatini, *Harvard Medical School, Boston, Massachusetts*

Synapse to Circuit II
Chairpersons: J. Kaplan, *Massachusetts General Hospital, Harvard Medical School, Boston;* G. Turrigiano, *Brandeis University, Waltham, Massachusetts*

Stem Cells, Regeneration, and Disease II
Chairpersons: Y. Zuo, *University of California, Santa Cruz;* S. Temple, *Albany Medical College, New York*

Special Lectures
T. Jessell, *HHMI/Columbia University, New York*
The Neurons and Networks of Spinal Motor Control
E. Marder, *Brandeis University, Massachusetts*
Compensation in Robust Network Performance

Axon to Synapse III
Chairpersons: M. Halloran, *University of Wisconsin, Madison;* T. Schwarz, *Children's Hospital, Boston, Massachusetts*

Relaxing on the lawn

Molecular Genetics of Aging

Sept. 28–Oct. 2   289 Participants

ARRANGED BY   **Steven Austad,** University of Texas Health Science Center, San Antonio
Judith Campisi, Buck Institute for Age Research, Lawrence Berkeley
     National Laboratory
David Sinclair, Harvard University Medical School

Aging is the largest single risk factor for developing a panoply of diseases, ranging from neurodegeneration to cancer. In recent years, enormous progress has been made in understanding how genetics and the environment collaborate to bring about the aging of organisms. Among the remarkable findings is the identification of molecular pathways that control aging in diverse species—from yeast to humans. This conference provided an intense forum for the latest results and emerging ideas in aging research.

The conference opened with a session on genetic variation—an exploration into why species, and particularly individuals within a species, age at different rates. A related session discussed genetic and epigenetic stability, which focused on the mechanisms that maintain the DNA sequence and its packaging (chromatin), how these entities change during aging, and how such changes affect organismal health and fitness. A subsequent session highlighted the role of mitochondria and energy metabolism in preserving organismal health and longevity, and the molecules that are crucial for optimal energy production and utilization. One session was devoted to cellular stress responses, and the intimate relationship between stress resistance and longevity. The conference then featured a session on stem cells, which emphasized recent findings on how these important sources of tissue repair and regeneration change with age, and how aging alters the tissue environment in which stem cells must function. Another session discussed protein homeostasis—how protein aggregates (which drive a number of age-related diseases) are formed and eliminated by specific genetic pathways. A session then focused on the power of genetic manipulations of conserved pathways to alter the rates and outcome

J. Sorrentino, C. Burd

of aging. The final session highlighted the latest findings on the role of nutrition and nutrient sensing in aging and discussed interventional strategies that are currently being developed.

The conference brought together scientists from diverse fields, mirroring the expanding multidisciplinarity of the field, and reflected the excitement and growing understanding of the processes that drive aging and the possibilities for developing interventions into both the aging process and the chronic diseases of aging.

This meeting was funded in part by the National Institute on Aging, a branch of the National Institutes of Health.

PROGRAM

Genetic Variation
Chairpersons: N. Barzilai, *Albert Einstein College of Medicine,*
Bronx, New York; R. Westendorp, *Leiden University Medical*
Center, The Netherlands

Genetic and Epigenetic Stability
Chairpersons: L. Donehower, *Baylor College of Medicine,*
Houston, Texas; J.P. Issa, *University of Texas M.D. Anderson*
Cancer Center, Houston

Oral Presentations from Abstracts
Chairpersons: S. Austad, *University of Texas Health Science Center, San Antonio;* J. Campisi, *Lawrence Berkeley National Laboratory, Buck Institute for Age Research, California;* D. Sinclair, *Harvard Medical School, Boston, Massachusetts*

Metabolism and Bioenergetics
Chairpersons: C. Mobbs, *Mount Sinai School of Medicine, New York;* R. De Cabo, *NIA, National Institutes of Health, Bethesda, Maryland*

Cellular and Organismal Stress Responses
Chairpersons: D. Peeper, *Netherlands Cancer Institute, Amsterdam;* S.-J. Lin, *University of California, Davis*

Tissue Maintenance/Stem Cells
Chairperson: G. Enikolopov, *Cold Spring Harbor Laboratory*

Protein Homeostasis
Chairpersons: N. Tavernakis, *Foundation for Research and Technology, Greece;* R. Morimoto, *Northwestern University, Evanston, Illinois*

Genetic Manipulations
Chairperson: G. Lithgow, *Buck Institute for Age Research, Novato, California*

Nutrient Sensing and Interventions
Chairpersons: B. Kennedy, *University of Washington, Seattle;* A. Dillin, *Salk Institute, La Jolla, California*

E. Jaehing, S.-J. Lin

N. Hartman, L. Zidek

M. Bonkowski, S. Gravina

H. Siegfried

Germ Cells

October 5–9	247 Participants
ARRANGED BY	**John Eppig,** The Jackson Laboratory
	Margaret Fuller, Stanford University School of Medicine
	Phillip Newmark, HHMI/University of Illinois at Urbana, Champaign

The tone for this meeting was set in the first session, with five speakers focusing on issues of germ cell specification, migration, and fate. As with all of the sessions, lively discussion followed each of the talks. Likewise, the diversity in the model systems used for study, ranging from flies to worms, sea urchin, fish, and mammals, highlighted deeply conserved emerging mechanisms. Session 2 focused on an area of particularly timely interest, Germ Line Stem Cells, a theme revisited with different emphasis throughout the meeting, particularly in Session 4, which focused on the switch from mitotic to meiotic cell divisions. Evolving concepts about the nature of the germ cell niche and the molecules driving stem cell dynamics provoked considerable discussion.

Poster Sessions (Sessions 3 and 6) presented studies on virtually all aspects of germ cell development in diverse model species. There was even a presentation of ovarian development in the plains viscacha with its unusual characteristics of absence of follicular atresia and massive numbers of ovulated eggs. Sessions 7 through 9 reported rapid advancement of our understanding of the regulation of meiosis and the oocyte-to-embryo transition. This was emphasized by studies on the role of small interfering RNAs and other posttranscriptional control mechanisms and the functions of maternal factors essential for early embryonic development.

P. Lasko, L. Cooley

There has been remarkable progress since the last Germ Cells meeting (in 2008). The evolution of new methods in molecular and genetic analyses, as well as concepts in control mechanisms such as small interfering RNA and molecular determinants of pluripotency, have greatly advanced the field of germ cell biology. Participants enjoyed breathtaking live-cell/organ imaging that provided unique perspectives on germ cell behavior and the possible functions of germ cell niches. The field looks forward to new applications of this exciting technology that will help shape our views of germ cell development. An international cast, including speakers from Japan, Australia, Taiwan, Germany, Switzerland, and the UK, participated in the meeting. There was general excitement about continued advancements that will affect broad basic and clinical fields of development, reproduction, and clinical sciences.

This meeting was funded in part by the National Institute of Child Health and Human Development, a branch of the National Institutes of Health; the Lalor Foundation; and the March of Dimes.

PROGRAM

Germ Cell Specification, Migration, and Fate
Chairperson: A. Spradling, *HHMI/Carnegie Institution, Baltimore, Maryland*

Germ Line Stem Cells
Chairperson: J. Kimble, *HHMI/University of Wisconsin, Madison*

Germ Line Stem Cells, Gonocyte Proliferation, and the Switch from Mitosis to Meiosis
Chairperson: B. Braun, *The Jackson Laboratory, Bar Harbor, Maine*

Transcriptional Control and Epigenetic Programming
Chairperson: H. Lin, *Yale University, New Haven, Connecticut*

Posttranscriptional Control and Small RNAs in Gametogenesis and Early Embryos
Chairperson: R. Lehmann, *HHMI/Skirball Institute, New York University School of Medicine, New York*

Meiosis
Chairperson: M.A. Handel, *The Jackson Laboratory, Bar Harbor, Maine*

Cell Biology of Germ Cells
Chairperson: D. Page, *HHMI/Massachusetts Institute of Technology, Cambridge*

Germ Line/Soma Communication and Sex Determination
Chairperson: E. Matunis, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

S. Hammond, M. Potok

B. Braun, D. Licatolosi

Mouse Development, Genetics, and Genomics

October 26–30   179 Participants

ARRANGED BY   **Kathryn Anderson,** Memorial Sloan-Kettering Cancer Institute
                   Haruhiko Koseki, RIKEN Research Center for Allergy & Immunology
                   Michael Shen, Columbia University Medical Center
                   William Skarnes, Wellcome Trust Sanger Institute

This 23rd annual meeting attracted a diverse group of scientists studying various molecular and genetic aspects of mammalian development. This meeting provided a format for the exchange of ideas and information, to discuss the latest research findings and technical advances toward the study of mammalian development and disease. The annual Rosa Beddington Lecture was given by Sally Dunwoodie on "Straightening out Notch," and Nobel laureate Mario Capecchi delivered a fascinating Keynote Lecture.

W. Skarnes, J. Lopez-Rios

The meeting included two poster sessions and eight oral sessions. The growing emphasis on genetics/genomics and on human disease models, already noted in previous years, continues, reflecting the increasing importance of the mouse as a model to investigate disease mechanisms, and the sophistication with which diseases can now be modeled and analyzed in this organism. After a long and illustrious history at Cold Spring Harbor, the Mouse meeting will now be moving elsewhere, in part a reflection of the enormous success of the mouse community in making this the central model for the study of human biology and disease, with sessions on mouse models at many of the other Cold Spring Harbor meetings, from cancer to neurodegenerative diseases and aging.

M. Manley, L. Naiche

M. Skamagki, D. Maatouk

PROGRAM

Human Disease and Cancer
Chairperson: G. Karsenty, *Columbia University, New York*

Organogenesis
Chairperson: K. Kaestner, *University of Pennsylvania Medical School, Philadelphia*

Technology
Chairperson: A. Nagy, *Samuel Lunenfeld Research Institute, Mt. Sinai Hospital, Toronto, Canada*

Patterning
Chairperson: P. Soriano, *Mount Sinai School of Medicine, New York*

Keynote Address
Chairperson: M. Capecchi, *University of Utah School of Medicine*

Neurobiology
Chairperson: S. Pfaff, *HHMI/The Salk Institute, La Jolla, California*

Epigenetics
Chairperson: T. Bestor, *Columbia University College of Physicians & Surgeons, New York*

Genetics and Genomics
Chairperson: J. Baker, *Stanford University, California*

Rosa Beddington Lecture
Chairperson: S.L. Dunwoodie, *Victor Chang Cardiac Research Institute, Sydney, Australia*

Germ Cells and Stem Cells
Chairperson: K. Hochedlinger, *Massachusetts General Hospital, Boston*

F. Spitz, P. Tschopp

H. Zong, M. Capecchi

Pharmacogenomics and Personalized Therapy

November 17–21   137 Participants

ARRANGED BY   **Hiltrud Brauch,** Ikp Stuttgart, Germany
Panagiotis Deloukas, Wellcome Trust Sanger Institute
Deanna Kroetz, University of California, San Francisco
Munir Pirmohamed, University of Liverpool
David Valle, Johns Hopkins University School of Medicine
Dick Weinshilboum, Mayo Medical School, Minnesota

This 8th annual meeting was a joint project of CSHL and the Wellcome Trust, funded in part by the National Institute of General Medical Sciences (NIGMS), one of the National Institutes of Health institutes. This series of meetings, held in alternating years on the Cold Spring Harbor Laboratory and Wellcome Trust Genome campuses, is designed to bring together scientists from disciplines that range from basic genomics to clinical medicine who are studying the role of inheritance in variation in drug response phenotypes, phenotypes that can range from life-threatening adverse drug reactions to lack of the desired therapeutic drug effect.

D. Weinshilboum, D. Valle

As a result, pharmacogenomics represents a major component of the movement toward "personalized" or "individualized" medicine. The opening session at the meeting began with a scientific presentation by Eric Green, director of NHGRI, followed by a presentation from Michel Eichelbaum, former director of the Dr. Margarete Fischer-Bosch Institute of Clinical Pharmacology in Stuttgart, Germany.

A major theme running through the meeting was the widespread application of genome-wide association studies and next-generation sequencing techniques to pharmacogenetic problems. The bottleneck posed by the explosive growth in the amount of data that can be generated with new genomic technologies was highlighted in sessions on translational bioinformatics and next-generation sequencing approaches. The importance of considering genetic variation in noncoding regions and structural variants was discussed by Peter Donnelly of the Wellcome Trust Centre for Human Genetics. Novel approaches to predicting the effects of genetic variation on protein function were presented in a session on genetic control of expression, and epigenetics and functional genomic applications to pharmacogenomics were also discussed as important areas for pharmacogenomics research. Pharmacoeconomic barriers to the application of pharmacogenomics to patient care illustrated the need for increased effort on the translation of pharmacogenomics findings.

The major theme that emerged from the meeting was the importance of rapid adoption of new genomic technologies and the need for increased translation of pharmacogenomics research findings into clinical settings to facilitate the optimization of drug therapy.

This meeting was funded in part by the National Institutes of General Medical Sciences, a branch of the National Institutes of Health.

PROGRAM

Opening Session

Translational Bioinformatics
Chairperson: E. Schadt, *Pacific Biosciences, Menlo Park, California*

Next-Generation Sequencing Approaches in Pharmacogenomics
Chairperson: D. Nickerson, *University of Washington, Seattle*

Genome-Wide Analyses and CNVs
Chairperson: P. Donnelly, *Wellcome Trust Centre for Human Genetics, Oxford, United Kingdom*

Genetic Control of Expression
Chairperson: S. Brenner, *University of California, Berkeley*

Epigenetics and Functional Genomics
Chairperson: M. Esteller, *Bellvitge Biomedical Research Institute, Barcelona, Spain*

ELSI, Pharmacoeconomic Issues, and Population-Based Pharmacogenetics
Chairperson: D. Veenstra, *University of Washington, Seattle*

A. Alfireule, P. Deloukas

M. Radonjic, B. Drögemüller, G. Wright

A. Frauman

Neurodegenerative Diseases: Biology and Therapeutics

December 1–4   134 Participants

ARRANGED BY   **Sam Gandy,** Mount Sinai School of Medicine
Virginia Lee, University of Pennsylvania School of Medicine
Jeffrey Rothstein, Johns Hopkins University School of Medicine

Up to one-half of those aged 85 years or older will develop debilitating degenerative disease of the central nervous system. These various diseases include Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS). Although these illnesses appear most commonly in the absence of obvious heritability or identifiable genetic mutations, it has been possible during the past 20 years to discover uncommon genetic mutations as well as risk-modifying DNA changes in some examples and predictable causative changes in others. From these findings, transgenic technology has rapidly led to the development of mouse, fruit fly, and nematode model systems that partially recapitulate the clinical abnormalities of the human diseases as well as some of the hallmark molecular and morphological pathology of the conditions.

In recent years, various molecular, biochemical, and cell-based screens have led to small-molecule peptides and oligonucleotide compounds that show promise in neurodegenerative disease models. Importantly, in the last year, the emergence of human cellular models, based on iPS (stem cell) technology, has become one of the most potentially important tools for disease pathogenesis and drug discovery. At this year's meeting in the biannual series, which started in 2000, numerous academic labs and commercial drug discovery organizations presented data on novel compounds, clinical trial results, and new druggable pathways for AD, PD, HD, ALS, spinal muscular atrophy, and spinocerebellar ataxia. As in previous years, the explicit goal of the meeting focused on facilitating the translation of "breakthrough" science into effective medicines.

V. Lee, J. Witkowski

For this meeting, platform sessions were organized around common technological themes. Chairpersons, invited speakers, and speakers selected from submitted abstracts were drawn from the academic and pharma sectors. The discussion of new, unpublished data was emphasized. Poster presentations also covered a wide range of neurodegenerative disease pathways, new animal and insect models, and novel therapeutic insights. Posters were displayed for an extended period during the meeting, and poster viewing was especially encouraged during the cocktail hours before the evening meals.

Topics in the 2010 meeting included monoclonal antibody and protein therapeutics for AD and ALS; biomarkers including imaging ligands and TDP-43 pathology; and RNA metabolism including splicing pathology, antisense modification of splicing, and RNA mapping. A number of investigators explored new intracellular trafficking pathways in AD models. The role of glial cells—oligodendroglia and astroglia—was highlighted including potential glial-centric therapeutics. New clinical trial results in AD were reviewed. At the prior meeting in 2008, the first new data on TDP-43 pathology was discussed. Now 2 years on, an explosion of science has developed around this mutation and large number of sporadic neurodegenerative diseases that appear to implicate TDP-43 pathogenesis, with in vitro and animal tools that may be useful for future drug discovery.

This meeting was funded in part by Merck Research Laboratories.

PROGRAM

Alzheimer's Clinical Trials Update
Chairperson: S. Gandy, *Mount Sinai School of Medicine, New York*

Biomarkers and Genetics
Chairperson: V. Lee, *University of Pennsylvania School of Medicine, Philadelphia*

RNA Metabolism in Neurodegenerative Disease
Chairperson: J. Rothstein, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Submitted Abstracts
Chairperson: S. Gandy, *Mount Sinai School of Medicine, New York*

Protein Sorting and Vps10 Proteins in Neurodegeneration
Chairperson: V. Lee, *University of Pennsylvania School of Medicine, Philadelphia*

Theme Stem Cells, Glia, and Synaptic Factors
Chairperson: J. Rothstein, *Johns Hopkins University School of Medicine, Baltimore, Maryland*

Drug Targets and Assays for Drugs
Chairperson: S. Gandy, *Mount Sinai School of Medicine, New York*

I. de Jong

A. Krainer, G. Schellenberg

R. Fisher

R. Darnell

Automated Imaging and High-Throughput Phenotyping

December 5–8   114 Participants

ARRANGED BY   **Philip Benfey,** Duke University
Anne Carpenter, Broad Institute of Harvard and Massachusetts Institute of Technology
Robert Waterston, University of Washington
Uwe Ohler, Duke University

This first meeting on Automated Imaging and High-Throughput Phenotyping was enormously successful. A total of 42 investigators presented in the six scientific sessions, with nearly 32 platform and poster presentations and 114 registered attendees. The six platform sessions and one poster session were marked by dynamic and enthusiastic exchanges of new results. In the scientific sessions, many audience members participated in the question and answer sessions, and the poster session was well attended.

Sessions at the meeting are listed below. Two additional sessions engaged the community in discussion: Future directions for the community as a whole, and Guidelines for databasing and publishing phenotyping data.

LemnaTec GmbH and Molecular Devices provided partial support for the meeting.

PROGRAM

Cellular Phenotyping
Chairperson: R. Waterston, *University of Washington, Seattle*

Imaging Technologies and Platforms
Chairperson: A. Carpenter, *Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge*

Developmental Phenotyping
Chairperson: F. Piano, *New York University, New York*

Future Directions

Stimulus/Response Phenotyping
Chairperson: U. Ohler, *Duke University, Durham, North Carolina*

Image Databasing and Knowledge Representation
Chairperson: P. Benfey, *Duke University, Durham, North Carolina*

Workshop on Databasing/Publication Guidelines

Organismal Phenotyping
Chairperson: S. Fraser, *California Institute of Technology, Pasadena*

A. Carpenter, P. Benfey

F. Piano, R. Waterston

Blood Brain Barrier Physiology

December 8–11   88 Participants

ARRANGED BY   **Roland J. Bainton,** University of California, San Francisco
Ben Barres, Stanford University
Richard Daneman, University of California, San Francisco

The second biotechnology conference addressed blood brain barrier (BBB) physiology. The conference encouraged new conceptual approaches and novel methods for our understanding of the neuroprotective physiologies of BBB structures, pairing genetics of model organism and associated biological methods with recent insights into vertebrate BBB physiology and development.

The conference fostered cross-disciplinary exchange of ideas and expertise between developmental and evolutionary biologists, vascular and BBB physiologists, and disease-oriented industry scientists interested in modifying or circumventing specific pathways of neuroprotection. Keynote talks on different aspects of BBB physiology were given by Kwang Sik Kim, Corey Nislow, and Guri Giaver. Topics addressed by the conference included: pathogenesis at the BBB, cellular constituents of the barrier, complexities of junctional complexes, xenobiotic protection, regulation of barrier physiology, and screening methodologies targeting barrier functions. As is traditional at Cold Spring Harbor meetings, selection of material for oral and poster presentation was made by the organizers and individual session chairs on the basis of scientific merit.

R. Bainton, B. Barres, R. Daneman

PROGRAM

Modeling of the BBB
Chairpersons: E. Shusta, *University of Wisconsin, Madison;* M. Jacobson, *University of California, San Francisco*

Model Organisms for BBB Analysis
Chairpersons: I. Blasig, *Leibniz-Institut for Molekulare Pharmakologie, Berlin, Germany;* H. Gelbard, *University of Rochester Medical Center, New York*

Keynote Address: Central Nervous System Infection and Blood-Brain Barrier (BBB): Lessons Learned from *E. coli* Translocation of the BBB
K.S. Kim, *Johns Hopkins Children's Center, Maryland*

Cellular and Molecular Regulation of the BBB
Chairpersons: E. Dejana, *Fondazione Ifom, Milano, Italy;* A. Reijerkerk, *Vu University Medical Center, Amsterdam, The Netherlands*

N. Saunders, S. Liddelow

Angiogenesis and BBB Development
Chairpersons: N. Saunders, *University of Melbourne, Parkville, Australia;* C. Gu, *Harvard Medical School, Boston, Massachusetts*

Disease States of the BBB
Chairpersons: K. Akassoglou, *Gladstone Institutes/University of California, San Francisco;* A. Flugel, *University Medical Center Göttingen, Germany*

Keynote Address
C. Nislow and G. Giaever, *University of Toronto, Canada*
Crossing Biological Barriers: Yeast and Worm Chemical Biology

Mechanisms of BBB Crossing
Chairpersons: B. Engelhardt, *University of Bern, Switzerland;* X. Nassif, *Inserm U570, Hopital Necker Enfants Malades, Paris, France*

M. Rengarajan, B. Dauwalder

COLD SPRING HARBOR LABORATORY/ WELLCOME TRUST CONFERENCES

This year marked the final year of the joint Cold Spring Harbor Laboratory/Wellcome Trust conference program at the Wellcome Trust Conference Centre in Hinxton, United Kingdom. Initiated in 2001, more than 3500 scientists have attended meetings on topics as diverse as genome informatics, host–pathogen interactions, and prion biology. The UK program also provided Cold Spring Harbor Laboratory with the opportunity to develop a number of conferences that alternated between the two sites, for example, the meeting on Pharmacogenomics that now alternates between the United States and Europe. The Wellcome Trust Conference team have now developed the tools and skills to manage the academic conference program independently, and we wish them well in their endeavors.

Aerial view over Francis Crick Auditorium and the Genome campus

Conference Hall

Wellcome Trust Conference Center; main center and Hinxton Hall

Computational Cell Biology

February 10–14   63 Participants

ARRANGED BY   **Benjamin Geiger,** Weizmann Institute
Edda Klipp, Max-Planck Institute for Molecular Genetics
Bela Novak, Oxford Centre for Integrative Systems Biology

The specific goal for this meeting was to foster fruitful and creative dialogue between experimental cell biologists and mathematical–computational modelers with common interests in the regulation of cell physiology. Emphasis was placed on areas of greatest current interest and importance, such as cell signaling, motility, cell proliferation and death, calcium dynamics, microbial physiology, and development. Sessions were also devoted to new experimental technologies and software developments.

Joint Cold Spring Harbor Laboratory/Wellcome Trust conferences at Hinxton are supported in part with funding courtesy of The Wellcome Trust.

PROGRAM

Keynote Addresses
J. Ferrell, *Stanford University, California*
Cooperative Phosphorylation in the Regulation of Wee1A
B. Scheres, *Utrecht University, The Netherlands*
Multilevel Analysis of Plant Development

Intracellular Signaling
Chairperson: M. White, *University of Liverpool, United Kingdom*

Tools and Software
Chairperson: N. Le Novère, *EMBL-European Bioinformatics Institute, Hinxton, United Kingdom*

Software Demo and Poster Session I

Self-Organization in Cells
Chairperson: B. Mulder, *FOM Institute AMOLF, Amsterdam, The Netherlands*

Cell Motility
Chairperson: F. Nédélec, *European Molecular Biology Laboratory, Heidelberg, Germany*

Software Demo and Poster Session II

Patterns in Development
Chairperson: J. Traas, *École Normale Supérieure de Lyon, France*

Cell Biophysics
Chairperson: K. Kruse, *Universität des Saarlandes, Germany*

Socializing at the meeting during coffee break

Systems Biology: Networks

August 11–15   108 Participants

ARRANGED BY   **Patrick Aloy,** Institute for Research Biomedicine, Spain
Anne-Claude Gavin, EMBL, Germany
Trey Ideker, University of California, San Diego
Marian Walhout, University of Massachussetts Medical School

The 2010 conference was again held at the Wellcome Trust Conference Centre on the Wellcome Trust Genome Campus in Hinxton, United Kingdom. Network Biology attempts to understand biology from the point of view of global and local systems properties of molecular networks. The conference addressed the structure, function, and dynamics of a variety of cellular networks such as genetic, transcriptional, posttranscriptional gene regulatory, protein–protein interaction, signaling, and metabolic networks. A central theme of the meeting was to understand how network properties relate to phenotypes, including human disease.

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PROGRAM

Gene Regulatory Networks I
Chairperson: L. Serrano, *Centre for Genomic Regulation, Barcelona, Spain*

Keynote Addresses
C. Boone, *University of Toronto, Toronto, Canada*
The Genetic Landscape of a Cell
E.M. Marcotte, *University of Texas, Austin*
Deaf Plants, Bleeding Yeast, and Other Surprising Disease Models from Deeply Conserved Gene Networks

Protein–Protein Interaction Networks
Chairperson: V. Cheung, *University of Pennsylvania, Philadelphia*

Genetic Networks
Chairperson: H. Zhu, *Johns Hopkins University, Baltimore, Maryland*

Network Medicine
Chairperson: P. Braun, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Gene Regulatory Networks II
Chairperson: G. Superti-Furga, *Austrian Academy of Sciences, Vienna, Austria*

Signaling Networks
Chairperson: A. Godzik, *Burnham Institute for Medical Research, La Jolla, California*

Metabolic Networks
Chairperson: D. Pe'er, *Columbia University, New York*

An animated discussion after one session

Infectious Disease Genomics and Global Health

September 12–15   86 Participants

ARRANGED BY   **Matthew Berriman,** Wellcome Trust Sanger Institute
Jane Carlton, New York University
Julian Parkhill, Wellcome Trust Sanger Institute
George Weinstock, Washington University School of Medicine

This fourth conference was held at the Wellcome Trust Conference Centre on the Wellcome Trust Genome Campus in Hinxton, United Kingdom. The meeting explored how the recent progress in host, pathogen, and vector genome data and other large data sets is changing the way we think about infectious diseases with an emphasis on global health issues.

Joint Cold Spring Harbor Laboratory/Wellcome Trust conferences at Hinxton are supported in part with funding courtesy of The Wellcome Trust.

PROGRAM

Keynote Address
D. Kwiatkowski, *Oxford University, Sanger Institute*

Epidemiology and Public Health
Chairpersons: C. Arias, *University of Texas Medical School, Houston*; P. Bejon, *Kemri Wellcome Trust Research Program, Kilifi, Kenya*

Viruses I
Chairpersons: R. Heyderman, *Malawi-Liverpool Wellcome Trust, Blantyre, Malawi*; P. Kellam, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Viruses II
Chairpersons: C. Simmons, *Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam*; L. Kramer, *Wadsworth Center, SUNY Albany, New York*

Population Genomics
Chairpersons: D. Conway, *Medical Research Council, The Gambia*; P. Keim, *Northern Arizona University, Flagstaff*

Bacteria
Chairpersons: N. Day, *Mahidol Oxford Tropical Medicine Research Unit (Moru), Bangkok, Thailand*; J. Vinetz, *University of California, San Diego*

Parasites and Vectors
Chairpersons: T. Anderson, *Southwest Foundation for Biomedical Research, San Antonio, Texas*; A. Das, *National Institute of Malaria Research (ICMR), New Delhi, India*

Coffee break

Genome Informatics

This conference focuses on large-scale genome informatics. Biology is an experimental science that is experiencing an explosion of new data. This requires biologists to increase the scale and sophistication in the information technology used for their research. The conference scope encompasses the management and the analysis of these data, such as whole-genome comparisons within and among species and strains, the analysis of results from high-throughput experiments to uncover cellular pathways and molecular interactions, and the design of effective algorithms to identify regulatory sequence motifs. It was the tenth Cold Spring Harbor Laboratory/Wellcome Trust Conference on Genome Informatics to be held at the Wellcome Trust Conference Centre on the Wellcome Trust Genome Campus in Hinxton, United Kingdom.

Joint Cold Spring Harbor Laboratory/Wellcome Trust conferences at Hinxton are supported in part with funding courtesy of The Wellcome Trust.

PROGRAM

Keynote Address: RNA Wikiproject—Community Annotation of RNA Families
A. Bateman, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Sequencing Pipelines and Assembly
Chairpersons: Z. Torok, *Astrid Research, Debrecen, Hungary*; I. Birol, *BC Cancer Agency, Vancouver, Canada*

Population and Statistical Genomics
Chairpersons: R. Durbin, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*; E. Margulies, *NIGRII, National Institutes of Health, Bethesda, Maryland*

Environmental and Medical Genomics
Chairpersons: T. Thorgeirsson, *University of California, Santa Cruz*; M. Clamp, *Bioteam, Middleton, Massachusetts*

Databases, Data Mining, Visualization, and Curation
Chairpersons: D. Dooling, *Washington University School of Medicine, St. Louis, Missouri*; M. Krzywinski, *BC Cancer Agency, Vancouver, Canada*

Epigenomics and Gene Regulation
Chairpersons: W. Noble, *University of Washington, Seattle*; Z. Weng, *University of Massachusetts Medical School, Worcester*

Comparative and Evolutionary Genomics
Chairpersons: S. Batzoglou, *Stanford University, California*; P. Flicek, *European Bioinformatics Institute, Hinxton, United Kingdom*

Sequencing and Gene Prediction
Chairpersons: A. Mortazavi, *California Institute of Technology, Pasadena*; L. Pachter, *University of California, Berkeley*

Discussing and looking over posters

POSTGRADUATE COURSES

The Postgraduate Courses program at Cold Spring Harbor Laboratory is aimed at meeting the special need for training in interdisciplinary subjects that are either so new or so specialized that universities do not adequately teach them. Our aim is to provide intensive study in the most recent developments and techniques in these subjects and to prepare students to enter directly into research in a particular area. To ensure up-to-date coverage of current research work, we bring together course faculty from many laboratories around the world and supplement this staff with a series of seminar speakers.

Protein Purification and Characterization

April 7–20

INSTRUCTORS
R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, M.D. Anderson Cancer Center/University of Texas, Houston
M. Marr, Brandeis University, Waltham, Massachusetts

ASSISTANTS
J. Cao, University of California, Los Angeles
M. Chambers, University of California, Los Angeles
R. Chumanov, University of Wisconsin, Madison
A. Esch, University of Wisconsin, Madison
Y.-U. Lee, M.D. Anderson Cancer Center, University of Texas, Houston
C. Olsen, Brandeis University, Waltham, Massachusetts
K. Pennington, Brandeis University, Waltham, Massachusetts
A. Ponce, University of California, Los Angeles
N. Thompson, University of Wisconsin, Madison
W. Turki-Judeh, University of California, Los Angeles

This course was for scientists who were not familiar with techniques of protein isolation and characterization. It was a rigorous program that included laboratory work all day and a lecture with discussion sessions every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue, (2) a sequence-specific DNA-binding protein, (3) a recombinant protein overexpressed in *Escherichia coli*, and (4) a membrane-bound receptor. A variety of bulk fractionation, electrophoretic, and chromatographic techniques included precipitation by salts, pH, and ionic polymers; ion exchange, gel filtration, hydrophobic interaction, and reverse phase chromatography; lectin affinity, ligand affinity, oligonucleotide affinity, and immunoaffinity chromatography; polyacrylamide gel electrophoresis, and electroblotting; and high-performance liquid chromatography. Procedures were presented for solubilizing proteins from inclusion bodies and refolding them into active monomeric forms. Methods of protein characterization were utilized to include immunological and biochemical assays, peptide mapping, amino acid analysis, protein sequencing, and mass spectrometry. Emphasis was placed on strategies of protein purification and characterization. Guest lecturers discussed protein structure, modification of proteins, methodologies for protein purification and characterization, and applications of protein biochemistry to cell and molecular biology and oncology.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Da Ros, M., M.Sc., University of Turku, Finland
Dantoft, T., M.S., Aarhus University, Faculty of Health Science, Denmark
Du, J., Ph.D., Medical College of Wisconsin, Milwaukee
Flores, G., B.S., University of Puerto Rico, San Juan
Foeger, N., B.A., Washington University in St. Louis, Missouri
Giniatullina, A., M.S., VU University Amsterdam, The Netherlands
Girgis, H., Ph.D., Massachusetts General Hospital, Boston
Jin, J.-K., B.S., University of Texas M.D. Anderson Cancer Center, Houston
Kalendova, A., M.S., Institute of Molecular Genetics AS CR, Prague, Czech Republic
Li, D., B.S., Massachusetts Institute of Technology, Cambridge
Lloyd, F., M.D., Indiana/Purdue University at Indianapolis, Indianapolis
Mosebi, S., Ph.D., Research Scientist at Mintek, Randburg, South Africa
Park, Y., Ph.D., University of Arkansas, Pine Bluff
Rak, R., B.S., Tel-Aviv University, Israel
Rincon, H., Ph.D., Fred Hutchinson Cancer Research Center, Seattle, Washington
Xu, J., Ph.D., University of California, Irvine

SEMINARS

Burgess, R., University of Wisconsin, Madison: Introduction to protein purification; RNA polymerase–Sigma factor interactions and use of LRET-based assays for drug discovery and biochemistry.
Chumanov, R., University of Wisconsin, Madison: Halo tag and purification of CARMI.
Courey, A., University of California, Los Angeles: System-wide analyses of Groucho and SUMO in *Drosophila* development.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Cadherin-11 in prostate cancer bone metastasis.
Marr, M., Brandeis University, Waltham, Massachusetts: Transcriptional and translational cellular responses to stress.
Thompson, N., University of Wisconsin, Madison: Immunoaffinity chromatography.

Cell and Developmental Biology of *Xenopus*

April 9–20

INSTRUCTORS	**R. Keller,** University of Virginia, Charlottesville
	K. Kroll, Washington University School of Medicine, St. Louis, Missouri
CO-INSTRUCTORS	**A. Sater,** University of Houston, Texas
	G. Thomsen, Stony Brook University, Stony Brook, New York
ASSISTANTS	**B. Dzamba,** University of Virginia, Charlottesville
	T. Nakayama, University of Virginia, Charlottesville
	K. Pfister, University of Virginia, Charlottesville

Xenopus is the leading vertebrate model for the study of gene function in development. The combination of lineage analysis, gene-knockout strategies, experimental manipulation of the embryo, and genomic/bioinformatic techniques makes it ideal for studies on the molecular control of embryo patterning, morphogenesis, and organogenesis. The course combined intensive laboratory training with daily lectures from recognized experts in the field. Students learned both emerging technologies and classical techniques to study gene function in *Xenopus* development. An important element was the informal interaction between students and course faculty. Technologies included oocyte and

embryo culture, lineage analysis and experimental manipulation of embryos, time-lapse imaging of morphogenesis, gain- and loss-of-function analyses using mRNAs and antisense oligos, whole-mount in situ hybridization, immunocytochemistry, RT-PCR, genomic/bioinformatic techniques, preparation of transgenic embryos, and use of *Xenopus tropicalis* for genetic analyses. This course was designed for those new to the *Xenopus* field, as well as for those wanting a refresher course in the emerging technologies. The course was open to investigators from all countries.

This course was supported with funds provided by the National Institute of Child Health and Human Development, the National Science Foundation, and the Howard Hughes Medical Institute.

PARTICIPANTS

Amin, N., Ph.D., University of North Carolina, Chapel Hill

Barnett, C., B.A., University of Alaska, Anchorage

Bertke, M., B.S., University of Notre Dame, Notre Dame, Indiana

Charney, R., B.A., University of California, Irvine, California

Cheng, W.-C., B.Sc., Kings College, London, United Kingdom

Gibb, N., B.Sc., University of Aberdeen, United Kingdom

Lloyd, R., Ph.D., Institute of Zoology, Zoological Society of London, United Kingdom

Messina, A., M.A., University of Trento, Italy

Nedelkovska, H., B.S., University of Rochester Medical Center, New York

Ploper, D., B.S., HHMI/University of California, Los Angeles

Riechert, S., M.S., Cancer Research, UK–London Research Institute, United Kingdom

Schneider, I., M.Sc., University of Hohenheim, Stuttgart, Germany

Stellabotte, F., Ph.D., House Ear Institute, Los Angeles, California

Walentek, P., M.S., University of Hohenheim, Stuttgart, Germany

SEMINARS

Christian, J., Oregon Health and Science University, Portland: TGF-β superfamily signaling in early *Xenopus* development.

Grainger, R., University of Virginia, Charlottesville: Genomic approaches for enhancer identification in *Xenopus*.

Harland, R., University of California, Berkeley: Biology of the organizer.

Houston, D., University of Iowa, Iowa City: Roles of localized maternal mRNAs in *Xenopus* axis formation.

Keller, R., University of Virginia, Charlottesville: Introduction to *Xenopus* morphogenesis.

Khokha, M., Yale University School of Medicine, New Haven, Connecticut: Introduction to *Xenopus tropicalis*.

Kintner, C., The Salk Institute, La Jolla, California: Cell biological analysis of early development.

Nakayama, T., University of Virginia, Charlottesville: Approaches for manipulating gene expression in *Xenopus*.

Vize, P., University of Calgary, Canada: Xenbase.

Zimmerman, L., National Institute for Medical Research, London, United Kingdom: Genetics in *Xenopus tropicalis*.

Workshop on Schizophrenia and Related Disorders

June 9–15

INSTRUCTORS   **A. Malhotra**, Zucker Hillside Hospital, Glen Oaks, New York
                                    A. Law, National Institute of Mental Health, NIH, Bethesda, Maryland

CO-INSTRUCTOR   **J. Hall**, University of Edinburgh, United Kingdom

ASSISTANT   **P. DeRosse,** Zucker Hillside Hospital, Glen Oaks, New York

This workshop provided students with the most current understanding of the molecular, cellular, and neural systems underlying the disturbances in brain function in these devastating illnesses. During the 7-day workshop, students learned about the clinical aspects of schizophrenia, schizoaffective disorder, and bipolar disorder, as well as explored in detail the genetic and neurobiological underpinnings of these complex psychiatric disorders. The Workshop included sessions focused on The Clinical Syndrome, Basic Neurobiology, Cognitive Neuroscience, Neuroimaging, Genetics and Genomics, Endophenotypes, and Gene Expression and Gene Modulation. In addition to hearing about the most recent research in these areas, controversial topics and challenges to basic assumptions in the field were explored and discussed. A diverse faculty brought the most up-to-date results and theories to the students, making this Workshop a valuable resource for young researchers starting out in this fast-moving and expansive field. Not only did it help them build the foundation for their future research, it also introduced them to many potential collaborators working to understand schizophrenia and similar disorders from different perspectives.

PARTICIPANTS

Balu, D., Ph.D., McLean Hospital, Belmont, Massachusetts

Becerril, K., B.S., Washington University, St. Louis, Missouri

Chang, W., B.S., University of California San Diego, La Jolla

Cui, X., Ph.D., Sigma Advanced Genetic Engineering (SAGE) Labs, St. Louis, Missouri

Dowd, E., B.S., Washington University, St. Louis, Missouri

Gallego, J., Ph.D., Zucker Hillside Hospital, Glen Oaks, New York

Kleinhaus, K., Ph.D., New York University School of Medicine

Maher, B., Ph.D., University of Connecticut, Storrs

Malavasi, E., B.S., University of Edinburgh, United Kingdom

Morris, H., Ph.D., Johns Hopkins Medical Institution, Baltimore, Maryland

Nucifora, F., Ph.D., Johns Hopkins School of Medicine, Baltimore, Maryland

Radhakrishnan, R., Ph.D., Yale University School of Medicine, New Haven, Connecticut

Smith, S., B.S., University of Missouri, Saint Louis

Stilo, S., Ph.D., Psychosis Clinical Academic Group, London, United Kingdom

Sussmann, J., Ph.D., Edinburgh University, Edinburgh, United Kingdom

Wang, X., Ph.D., University of Southern California, Los Angeles

Ward, K., B.S., Pfizer Global Research and Development, Groton, Connecticut

Woods, G., B.S., University of California, Davis

Zhang, J., Ph.D., Zucker Hillside Hospital, Glen Oaks, New York

Zhu, S., Ph.D., Johns Hopkins University School of Medicine, Baltimore, Maryland

SEMINARS

Abi-Dargham, A., Columbia University, New York: PET imaging in schizophrenia.

Akbarian, S., University of Massachusetts, Medical School, Worcester: Epigenetics in schizophrenia.

Calicott, J., National Institute of Mental Health, NIH, Bethesda, Maryland: Functional MR in schizophrenia.

Cornblatt, B., Zucker Hillside Hospital, Glen Oaks, New York: Cognitive and psychosocial risk factors.

DeRosse, P., Zucker Hillside Hospital, Glen Oaks, New York: Introduction to the clinical syndrome.

Fletcher, P., University of Cambridge, United Kingdom: A cognitive neuroscientific model of the positive symptoms of schizophrenia.

Hall, J., University of Edinburgh, United Kingdom: Cognition of schizophrenia and imaging genomics.

Hyde, T., National Institute of Mental Health, NIH, Bethesda, Maryland: Postmortem gene expression in neurodevelopment.

Jarskog, F., Columbia University, New York: Treatment strategies.

Law, A., National Institute of Mental Health, Bethesda, Maryland: NRG/ErbB signaling and risk for schizophrenia: The genetics of gene regulation and function.

Lawrie, S., University of Edinburgh, United Kingdom: Structural imaging in schizophrenia.

Lewis, D., University of Pittsburgh, Pennsylvania: Cortical GABA systems in schizophrenia.

Li, B., Cold Spring Harbor Laboratory: Glutamatergic synapse and its receptors: From cognitive function to psychiatric disorder.

Malhotra, A., Zucker Hillside Hospital, Glen Oaks, New York: Pharmacogenomics in schizophrenia.

Moghaddam, B., University of Pittsburgh, Pennsylvania: The glutamatergic system.

O'Donnell, P., University of Maryland, Baltimore: The dopaminergic system.

O'Donovan, M., University of Wales, Cardiff, United Kingdom: GWAS and candidate genes in schizophrenia.

Porteous, D., University of Edinburgh, United Kingdom: Common and rare variants in schizophrenia: The DISC1 example.

Sebat, J., University of California, San Diego: Rare variants in schizophrenia.

Song, H., Johns Hopkins University, Baltimore, Maryland: Neurogenesis and iPS in schizophrenia.

Susser, E., Columbia University, New York: Epidemiology and environmental risk factors.

Weinberger, D., National Institute of Mental Health, Bethesda, Maryland: Intermediate phenotypes and schizophrenia-associated genes.

Advanced Bacterial Genetics

June 9–29

INSTRUCTORS   **J. Kirby,** University of Iowa, Iowa City
S. Lovett, Brandeis University, Waltham, Massachusetts
A. Segall, San Diego State University, San Diego, California

ASSISTANTS   **K. Elliott,** University of Georgia, Westminster, South Carolina
J. Rostron, University of Iowa, Iowa City
C. Weitzel, Brandeis University, Waltham, Massachusetts
J. Willett, University of Iowa, Iowa City

This course presented logic and methods used in the genetic dissection of complex biological processes in diverse bacteria. Laboratory methods included classical mutagenesis using transposons, mutator strains, and chemical and physical mutagens; detection and quantitation of gene expression changes using various reporter genes and real-time PCR; the mapping of mutations using genetic and physical techniques; modern approaches to the generation and analysis of targeted gene disruptions and fusions using PCR and cloning methods; epitope insertion mutagenesis; site-directed mutagenesis; and fluorescence microscopy. Key components of the course were the use of sophisti-

cated genetic methods in the analysis of model bacteria (including *Escherichia coli*, *Salmonella*, *Bacillus subtilis*, and *Myxococcus xanthus*) and the use of the wealth of new genomic sequence information to motivate these methods. Invited lecturers presented various genetic approaches to study bacterial mechanisms of metabolism, development, and pathogenesis.

PARTICIPANTS

Arnoldini, M., M.Sc., ETH Zurich, Switzerland

Barrett, E., B.S., Dartmouth College, Hanover, New Hampshire

Brolund, A., M.Sc., Karolinska Institute, Stockholm, Sweden

Cady, K., B.S., Dartmouth Medical School, Lebanon, New Hampshire

Carpino, J., B.S., Queens College and CUNY Graduate Center, Flushing, New York

Hao, L., M.S., University of Wisconsin, Madison

Jun, S., B.Sc., Harvard University, Cambridge, Massachusetts

Kuehn, S., Ph.D., The Rockefeller University, New York

Lazova, M., M.Sc., FOM Institute AMOLF, Amsterdam, The Netherlands

Lewis, E., B.S., University of California, Irvine

Liu, X., Ph.D., Harvard Medical School, Boston, Massachusetts

Rae, R., B.S., Max-Planck Institute for Developmental Biology, Tuebingen, Germany

Rodriguez Esperon, M., B.S., Institute De Invest. Clemente Estable, Montevideo, Uruguay

Sankararaman, S., Ph.D., University of California, Berkeley

Shanahan, E., B.Sc., Centenary Institute University of Sydney, Camperdown, Australia

Viburiene, R., M.S., University of Oslo, Norway

SEMINARS

Edwards, R., San Diego State University, California: Using genomics and metagenomics to understand the world around us.

Hughes, D., Uppsala University, Sweden: Bacterial mutants selected to cope with stressful growth conditions.

Kirby, J., University of Iowa, Iowa City: Chemosensory regulation of biofilm formation.

Laub, M., Massachusetts Institute of Technology/HHMI, Cambridge: Specificity and evolution of bacterial signaling pathways.

Lazazzera, B., University of California, Los Angeles: Identifying pathways to control surface-adhered biofilm formation.

Lovett, S., Brandeis University, Waltham, Massachusetts: Replication gap repair.

Rohwer, F., San Diego State University, California: Human virome.

Rudner, D., Harvard Medical School, Boston, Massachusetts: Regulation of essential cell biological processes during bacterial differentiator.

Segall, A., San Diego State University, California: Inhibitors of DNA repair and the serendipity of science.

Storz, G., National Institute of Child Health and Human Development, Bethesda, Maryland: The genes that were missed.

Whitaker, R., University of Illinois, Urbana: Dynamics of host–virus interactions across space and time.

Yildiz, F., University of California, Santa Cruz: Analysis of biofilm formation in *Vibrio cholerae*.

Ion Channel Physiology

INSTRUCTORS　　**N. Golding,** University of Texas, Austin
A. Lee, University of Iowa, Iowa City
M. Nolan, University of Edinburgh, United Kingdom

ASSISTANTS　　**C. Bladen,** University of Calgary, Alberta, Canada
K. Buchanan, University College London, United Kingdom
P. Dodson, University of Edinburgh, United Kingdom
A. Graves, Northwestern University, Evanston, Illinois
K. Hansen, Emory University, Atlanta, Georgia
P. Kammermeier, University of Rochester, New York
S. Khurana, University of Texas, Austin
L. Scott, University of Texas, Austin
K. Vance, Emory University, Atlanta, Georgia

The primary goal of this course was to investigate, through lectures and laboratory work, the properties of ion channels that allow neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitters at central and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) respond to neuromodulators with changes in functional properties, or (4) are developmentally required and regulated. The research interests of guest lecturers reflected these areas of emphasis.

The laboratory component of the course introduced students to electrophysiological approaches for the study of ion channels in their native environments. Hands-on exercises included patch-clamp recording of ion channel activity in acutely isolated or cultured cells or neurons in brain slice preparations. Different recording configurations were used (e.g., whole-cell, cell-free, and nucleated patches) to examine macroscopic or single-channel activity. Similarly, various methods of ligand and drug application were demonstrated. The advantages and disadvantages of each method, preparation, and recording technique were considered in relation to the specific scientific questions being asked. Admissions priority was given to students and postdocs with a demonstrated interest and specific plans, as well as a supportive environment to apply these techniques to a defined problem.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Altimimi, H., B.S., McGill University, Montreal, Canada

Gavin, C., B.A., The Scripps Research Insitute, Florida Campus, Jupiter

Goodfellow, N., B.S., University of Toronto, Canada

Gutnisky, D., Ph.D., Janelia Farm-HHMI, Ashburn, Virginia

Kalia, J., M.S., National Institutes of Health, Bethesda, Maryland

Kim, S.E., M.S., The Scripps Research Institute, La Jolla, California

Kuo, C., Ph.D., Duke University Medical Center, Durham, North Carolina

Madry, C., Ph.D., University College London, United Kingdom

Moscato, E., B.A., University of Pennsylvania, Philadelphia

Schoonover, C., Ph.D., Columbia University, New York

Surmeli, G., B.S., Columbia University, New York

Tauchmann, M., B.A., Arizona State University, Tempe

SEMINARS

Deisseroth, K., Stanford University, Stanford, California: Channel engineering.

Dolphin, A., University College London, United Kingdom: Voltage-gated Ca^{2+} channels. The $\alpha2\delta$ subunits of voltage-gated calcium channels: Involvement in disease and target site for drugs.

Dudman, J., Janelia Farm Research Campus, Ashburn, Virginia: Hyperpolarization-activated channels.

Farrant, M., University College London, United Kingdom: Inhibitory synaptic transmission and differential activation of $GABA_A$ receptor subtypes.

Golding, N., University of Texas, Austin: Synaptic integration and integrative properties for auditory temporal coding.

Jan, L., University of California, San Francisco: K^+ channels and Ca^+-activated C1-channels.

Kaczmarek, L., Yale University School of Medicine, New Haven, Connecticut: Ion channel modulation. Regulation of neuronal timing by short-term and long-term channel modulation.

Larsson, P., University of Miami, Florida: Voltage-gated K^+ channels and molecular mechanisms of voltage-gated proton channels.

Raman, I., Northwestern University, Evanston, Illinois: Na channels. Biophysics and physiology. Sodium currents specializations in spontaneous spiking and cerebellar signaling.

Sjostrom, J., University College London, United Kingdom: Plasticity and LTP. Spike-timing-dependent plasticity in neocortex.

Molecular Embryology of the Mouse

June 9–29

INSTRUCTORS
K. Hadjantonakis, Memorial Sloan-Kettering Cancer Institute, New York
L. Pevny, University of North Carolina, Chapel Hill
J. Rivera-Perez, University of Massachusetts Medical School, Worcester

ASSISTANTS
D. Escalante-Alcalde, Institute of Cellular Physiology–UNAM, Distrito Federal, CP, Mexico
A. Ferrer-Vaquer, Memorial Sloan-Kettering Cancer Institute, New York
D. Matsushima, University of North Carolina, Chapel Hill
G. Tortelote, University of Massachusetts Medical School, Worcester

This intensive laboratory and lecture course was designed for biologists interested in applying their expertise to the study of mouse development. Lectures provided the conceptual basis for contemporary research in mouse embryogenesis and organogenesis, and laboratory practicals provided extensive hands-on introduction to mouse embryo analysis. Experimental techniques covered included in vitro culture and manipulation of pre- and postimplantation embryos, embryo transfer, culture and genetic manipulation of embryonic stem cells, production of chimeras by embryo aggregation and by ES cell injection, and transgenesis by pronuclear microinjection. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including techniques of histology, in situ hybridization, immunohistochemistry, skeletal preparation, organ culture, and tissue recombination. The course also introduced the generation of iPS cells (induced pluripotent cells) and time-lapse microscopy of early gastrulation embryos and organ cultures (eye, kidney, and gut).

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Akinci, U., Ph.D., Erasmus Medical Center, Rotterdam, The
  Netherlands
Aylor, D., Ph.D., University of North Carolina, Chapel Hill
Azzoni, E., M.S., H. San Raffaele–DIBIT, Milan, Italy
Bezzi, M., M.D., Institute of Molecular and Cell Biology,
  Singapore
Chang, C., M.S., The Rockefeller University, New York
Cooper, L., Ph.D., University of Illinois, Urbana
Goetz, R., Dipl., University of Freiburg, Germany
Günesdogan, U., Dipl., Max-Planck Institute for Biophysical
  Chemistry, Göttingen, Germany

Lim, C., Ph.D., Institute of Medical Biology, Singapore
Muta, Y., Ph.D., RIKEN, Kobe, Japan
Peyrot, S., Ph.D., University of Texas, Austin
Ryall, J., Ph.D., National Institutes of Health, Bethesda,
  Maryland
Vagin, V., Ph.D., Cold Spring Harbor Laboratory
Wat, M., B.S., Baylor College of Medicine, Houston,
  Texas

SEMINARS

Anderson, K., Memorial Sloan-Kettering Cancer Institute,
  New York: ENU screens and signaling pathways.
Behringer, R., The University of Texas/M.D. Anderson Cancer
  Center: Transgenics and Evo-Devo.
Capel, B., Duke University Medical Center, Durham, North
  Carolina: Germ cells and the transcriptional network in the
  gonad.
Chapman, D., University of Pittsburgh, Pennsylvania:
  Somitogenesis.
Conlon, F., University of North Carolina, Chapel Hill: Heart
  development.
Costantini, F., Columbia University, College of Physicians &
  Surgeons, New York: Kidney development.
Dickinson, M., Baylor College of Medicine, Houston, Texas:
  Cardiovascular function and imaging.
Eggenschwiler, J., Princeton University, New Jersey: Genetic
  dissection of neural tube development.
Epstein, D., University of Pennsylvania, Philadelphia: Early
  neural development.
Hadjantonakis, K., Memorial Sloan-Kettering Cancer
  Institute, New York: Imaging early embryo morphogenesis.
Hochedlinger, K., Masschusetts General Hospital, Boston:
  Nuclear reprogramming and iPS cells.
Hogan, B., Duke University Medical Center, Durham, North
  Carolina: Lung development and progenitor cells.
Johnson, R., Anderson Cancer Center, Houston, Texas: Limb
  development and hippo signaling.
Joyner, A., Memorial Sloan-Kettering Cancer Institute, New
  York: Patterning and fate mapping of the neural system.
Lovell-Badge, R., MRC National Institute for Medical
  Research, London, United Kingdom: Primary sex
  orientation.

Magnuson, T., University of North Carolina, Chapel Hill:
  Epigenetics.
Nagy, A., Samuel Lunenfeld Research Institute, Toronto,
  Canada: ES cells, chimeras, and recombinases.
Papioannou, V., Columbia University, New York: The mouse
  as a model system.
Pevny, L., University of North Carolina, Chapel Hill:
  Transcriptional regulation of retinogenesis.
Rivera, J., University of Massachusetts Medical School,
  Worcester: Lineage analysis and early postimplantation
  development.
Robertson, E., University of Oxford, United Kingdom: Nodal
  signaling and axial development.
Shen, M., Columbia Unversity Medical Center, New York:
  Prostate development, stem cells, and cancer.
Solter, D., Institute of Medical Biology, Singapore, Singapore:
  Preimplantation development.
Soriano, P., Mount Sinai School of Medicine, New York: Gene
  trapping and signaling pathways.
Sussel, L., Columbia University, New York: Pancreas
  development.
Takahashi, J., HHMI/University of Texas Southwestern
  Medical Center, Dallas: Circadian rhythms.
Tam, P., Children's Medical Research Institute, Westmead,
  Sydney, Australia: Gastrulation.
Threadgill, D., North Carolina State University, Raleigh:
  Mouse genetics.
Trainor, P., Stowers Institute for Medical Research, Kansas
  City, Missouri: The neural crest in craniofacial development,
  evolution, and disease.
Turnbull, D., New York University Medical Center, New York:
  Imaging modailities and applications: Ultrasound and MRI.

Integrative Statistical Analysis of Genome-Scale Data

INSTRUCTORS   **H. Bussemaker,** Columbia University, New York
               V. Carey, Harvard University, Boston, Massachusetts
               M. Reimers, Virginia Commonwealth University, Richmond

ASSISTANTS   **N. Altman,** Penn State, University Park
               S. Bekiranov, University of Virginia, Charlottesville
               M. Figueroa, Weill Cornell Medical College, New York
               T. Guennel, Virginia Commonwealth University, Richmond
               A. Lazarovici, Columbia University, New York
               T. Riley, Columbia University, New York

Genome-scale data sets are a central feature of modern biological research. Experimental and computational biologists who want to get the most from their data sets need to have a firm grasp of statistical and analytical methodology. This course was designed to build competence in quantitative methods for the analysis of high-throughput molecular biology data. Detailed lectures and presentations by guest speakers in the morning and evening were combined with hands-on computer tutorials in the afternoon, in which the methods covered in the lectures were applied to actual high-throughput data for yeast and humans. Students were assumed to have a basic familiarity with the R programming language at the start of the course.

PARTICIPANTS

Bartonicek, N., B.S., European Molecular Biology Laboratories, Cambridge, United Kingdom

Berko, E., B.A., Albert Einstein College of Medicine, Bronx, New York

Cai, Y., M.S., Cold Spring Harbor Laboratory

Cho, M., M.D., Brigham & Women's Hospital, Boston, Massachusetts

Fendler, B., Ph.D., Cold Spring Harbor Laboratory

Fertig, E., Ph.D., Johns Hopkins University, Baltimore, Maryland

Grant, J., Ph.D., University of Alberta, Edmonton, Canada

Jonsson, P., M.S., University of Houston, Texas

Krupka, M., B.A., Albert Einstein College of Medicine, Bronx, New York

Liang, W., Ph.D., Translational Genomics Research Institute, Phoenix, Arizona

Mandal, A., B.Sc., Institute of Genomics & Integrative Biology, Delhi, India

Matarin, M., B.S., ION/UCL, London, United Kingdom

Mayfield, J., Ph.D., Duke University, Durham, North Carolina

Muller, J., M.S., A*Star, Singapore

Obholzer, N., B.S., Harvard Medical School, Boston, Massachusetts

Pereira, J., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania

Rosenbloom, K., B.A., University of California, Santa Cruz

Ross, E., M.B.A., Howard Hughes Medical Institute, Salt Lake City, Utah

Sorokin, E., B.A., University of Wisconsin, Madison

Stokowy, T., M.Sc., Silesian University of Technology, Gilwice, Poland

Wang, C., M.S., Uppsala University, Sweden

Wee, C., B.Sc., Temasek Lifesciences Laboratory, Singapore

Yuen, R., B.Sc., Child & Family Research Institute, Vancouver, Canada

Zhang, W., B.Sc., Roswell Park Cancer Institute, Buffalo, New York

SEMINARS

Altman, N., Penn State, University Park: Multiple comparisons: Concepts and methods and experimental design and basic statistical ideas. Exploratory data analysis and differential expression using RNA-Seq data.

Bekiranov, S., University of Virginia, Charlottesville: Physics of hybridization. High-throughput sequencing technology.

Bravo Corrado, H., Johns Hopkins University, Baltimore, Maryland: Base calling.

Bussemaker, H., Columbia University, New York: Gene-set based analysis of functional genomics data. Representations of protein-DNA-binding specificity I. Representations of protein-DNA-binding specificity II. Algorithms for de novo motif discovery. Linear modeling of transcriptional regulation.

Carey, V., Harvard University, Boston, Massachusetts: Bioconductor infrastructure for NGS.

Davis, S., National Institutes of Health, Bethesda, Maryland: Methylation and copy-number methods and integrated analysis of the NCI60.

Futcher, B., Stony Brook University, Stony Brook, New York: Lessons learned from a successful microarray experiment.

Gottardo, R., Clinical Research Institute of Montreal, Quebec, Canada: ChIP-chip data analysis. Research seminar.

Horvath, S., University of California, Los Angeles: Cancer classification. Research seminar. Cocorrelation analysis.

Liu, S., Harvard University, Cambridge, Massachusetts: ChIP-chip and ChIP-Seq analysis.

Lucito, R., Cold Spring Harbor Laboratory: Methylation and copy-number analysis.

Reimers, M., Virginia Commonwealth University, Richmond: Biases in RNA-Seq data. Integrated analysis of the NCI60.

Taub, M., Johns Hopkins University, Baltimore, Maryland: Intro to RNA-Seq. Processing RNA-Seq data. Biases in RNA-Seq data.

Workshop on Circuit and Molecular Architecture of the Vertebrate Brain

INSTRUCTORS
Z.J. Huang, Cold Spring Harbor Laboratory
P. Mitra, Cold Spring Harbor Laboratory
K. Rockland, Massachusetts Institute of Technology, Cambridge and
    RIKEN Brain Science Institute, Japan

ASSISTANTS
J. Hahn, University of Southern California, Los Angeles
V. Pinskiy, Cold Spring Harbor Laboratory
N. Wall, Salk Institute for Biological Studies, La Jolla, California
H. Wang, Cold Spring Harbor Laboratory

In comparison with complete reference genomes now available for multiple species, our knowledge about the neuronal and circuit architecture of the vertebrate nervous systems is relatively sparse. However, this situation is rapidly changing, enabled by technical advances as well as resurgent and widespread interest in the neuroscientific community in mapping out neural circuitry at unprecedented scales, ranging from the reconstruction of local microcircuits to the mapping of brain-wide mesocircuits. This circuit architecture naturally and logically complements the molecular architecture as delineated by the mapping of brain-wide gene expression patterns. Experimental efforts are under way in multiple species, promising to advance our knowledge of the wiring logic of the vertebrate brain. This fundamentally impacts on our understanding of brain function and evolution and also has an essential role in understanding pathological changes in circuitry that underlie neurological and neuropsychiatric disorders.

This new workshop brought together classical neuroanatomical approaches along with the new techniques that are enabling a new generation of neuroanatomical research into the circuit and molecular architecture of the vertebrate brain. The workshop had three main components: classical, mo-

lecular, and computational neuroanatomy. An experimental component of the workshop involved gross dissection (sheep brain); microscopic viewing of glass histology slides (sets of 20 slides per student); and injection-based tract tracing in the mouse, using classical and viral tracer substances, in wild-type and transgenic mice. Lectures covered classical (tracer injections, sectioning, histochemistry, imaging) and molecular (genetic engineering of mice as well as viral tracers, optogenetic probing of circuits) techniques. Material was presented by simultaneous viewing slides under light microscopy as well as digital images, including an in-depth orientation to Internet resources. Anatomical topics included specific circuits and pathways, molecular topics included development and cell-type specificity. The computational component involved hands-on algorithmic analysis and interpretation of digital neuroanatomical data sets, from both EM and light microscopy, and discussion of 3-0 brain morphing and enregistering of disparate date sets to a common reference. Species covered included rodents and human and nonhuman primates, with special lectures on other vertebrate lineages.

The course was supported by funds provided through Cold Spring Harbor Laboratory.

PARTICIPANTS

Beier, K., B.S., Harvard Medical School, Boston, Massachusetts
Bogart, L., B.S., Harvard University, Boston, Massachusetts
Bressan, D., M.Sc., Cold Spring Harbor Laboratory
Harrison, T., B.Sc., University of British Columbia, Vancouver, Canada
Laramee, M.-E., M.S., Université du Québec á Trois-Riviéres, Canada
McCarren, H., B.S., University of Pennsylvania, Philadelphia

Morgan, J., Ph.D., University of California, Davis, Sacramento
Takashima, Y., Ph.D., Carnegie Mellon University, Pittsburgh, Pennsylvania
Vannini, E., B.S., Scuola Normale Superiore, C.N.R., Pisa, Italy
Wolf, L., Ph.D., Tel-Aviv University, Israel
Yu, H.-H., Ph.D., Monash University, Clayton, Australia
Zhang, X., B.S., National Institutes of Biological Sciences, Beijing, China

SEMINARS

Allman, J., California Institute of Technology, Pasdena: The von economo Neurons (VENs) in fronto-insular and anterior cingulate cortex in apes and humans.
Amaral, D., University of California, Davis: The organization of the nonhuman primate and human hippocampal formation.
Bohland, J., Boston University, Massachusetts: An overview of image analysis challenges in high-throughput neuroanatomy. Imaging processing tutorial.
Cardona, A., University of Zurich, Switzerland: Neuronal reconstruction tutorial with Fiji open source software.
Chklovskii, D.B., HHMI/Janelia Farm, Ashburn, Virginia: Wiring principles and/or EM reconstruction pipeline.
Deisseroth, K., Stanford University, California: Optogenetics: development application.
Fischl, B., Martinos Center for Biomedical Imaging/MGH, Boston, Massachusetts: Introduction to MRI: What you can measure. Introduction to computational neuroanatomy using MRI.
Greenspan, R., The Neurosciences Institute, La Jolla, California: Invertebrate neuroanatomy.
Hawrylycz, M., Allen Institute for Brain Science, Seattle, Washington: Computational neuroanatomy of gene expression. Tutorial on Allen Institute tools.
Helmstaedter, M., Max-Planck Institute for Medical Research, Heidelberg, Germany: Dense circuit reconstruction from blockface electron microscopy data. EM reconstruction tutorial.

Henkelman, M., University of Toronto, Canada: Magnetic resonance imaging and computer analysis for mouse neuroanatomy.
Huang, Z.J., Cold Spring Harbor Laboratory: Genetic dissection of neural circuits in mouse brain. Introductory remarks: Why is molecular genetics useful in systems neuroscience.
Jeromin, A., Banyan Biomarkers, Inc., Alachua, Florida: High-throughput brain mapping: Projects and approaches. Neuroanatomy pipeline tour.
Karten, H., University of California, San Diego, La Jolla: Evolutionary origins of mammalian neocortex.
Kleinfeld, D., and Karten, H., University of California, San Diego, La Jolla: Introduction to wide-field and digital imaging. Brain image databases. Optical sectioning and 3D reconstruction.
Lee, J.H., University of California, Los Angeles: Optogenetic stimulation and fMRI.
Li, B., Cold Spring Harbor Laboratory: Synapse, circuit, and psychiatric disorders.
Martin, K., University of Zurich, Switzerland, Mapping the matrix.
Mello, C., Oregon Health and Science University, Portland: Gene expression in zebra finch.
Mitra, P., Cold Spring Harbor Laboratory: Course introduction.
Osten, P., Cold Spring Harbor Laboratory: Viral tracers.
Peterson, D., Rosalind Franklin University of Medicine and

Science, North Chicago, Illinois: Quantitative histology: Introduction to design-based stereology.

Pinskiy, V., Cold Spring Harbor Laboratory and Jeromin, A., Banyan Biomarkers, Inc., Alachua, Florida: Neuroanatomy pipeline tour.

Rockland, K., Massachusetts Institute of Technology, and RIKEN Brain Science Institute, Cambridge: Introduction to experimental techniques (stains and example connections). Gross neuroanatomy lab. Histology Lab I. Histology Lab II. PPT.

Rosa, M., Monash University, Victoria, Australia: Toward a computational atlas of the primate cerebral cortex and its connections.

Safdieh, J., Weill Cornell Medical College, New York: General organization of the human central and peripheral nervous system. Clinical case studies.

Saper, C., Harvard Medical School, Boston, Massachusetts: Techniques using thermoregulation as a platform.

Sestan, N., Yale University, New Haven, Connecticut: Molecular control of cortical projection neuron identity and connectivity.

Swanson, L., University of Southern California, Los Angeles: Studying long connections in the nervous system: Past, present, and future.

Wall, N., Salk Institute for Biological Studies, La Jolla, California: Modified rabies virus as a retrograde transsynaptic tracer and means for genetically targeting neuronal circuits.

Witter, M., Norwegian University of Science and Technology, Trondheim, Norway: Modern nueroanatomy applied to study the entorhinal-hippocampal network.

Xiaoxia, C., Sigma Advanced Genetic Engineering (SAGE) Labs, St. Louis, Missouri: Genetic engineering of the rat genome using zinc finger nucleases.

Zeng, H., Allen Institute for Brain Science, Seattle, Washington: Mouse genetic tool kit for a genetically based connectional atlas.

Computational Neuroscience: Vision

INSTRUCTORS **G. Boynton,** University of Washington, Seattle
G. Horwitz, University of Washington, Seattle
S. Treue, German Primate Center, Göttingen, Germany

ASSISTANTS **M. Cohen,** Harvard Medical School, Cambridge, Massachusetts
R. Kiani, HHMI/Stanford University School of Medicine, California

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.

PARTICIPANTS

Anton-Erxleben, K., Ph.D., New York University, New York
Cicmil, N., B.S., University of Oxford, United Kingdom
Czuba, T., B.S., University of Texas, Austin
Dold, H., B.S., Berlin Institute of Technology, Germany
Ernst, Z., B.S., University of Washington, Seattle

Freeman, J., B.S., New York University, New York
Heilbronner, S., B.S., Duke University Medical Center, Durham, North Carolina
Jepson, L., B.S., Salk Institute for Biological Studies, La Jolla, California

Marshel, J., B.S., Salk Institute for Biological Studies, La Jolla, California

Moeeny, A., Ph.D., National Institutes of Health, Bethesda, Maryland

Morgenstern, Y., B.S., York University, Toronto, Canada

Ni, A., B.S., Harvard Medical School, Boston, Massachusetts

Putzeys, T., B.S., University of Leuven, Belgium

Said, C., B.S., Princeton University, New Jersey

Saproo, S., B.S., University of California, San Diego, La Jolla

Scharff, A., B.S., University of Washington, Seattle

Scholvinck, M., Ph.D., University College London, United Kingdom

Smolyanskaya, A., B.S., Harvard Medical School, Boston, Massachusetts

Tan, C., B.S., Massachusetts Institute of Technology, Cambridge

Twarog, N., B.S., Massachusetts Institute of Technology, Cambridge

Wang, H., B.S., New York University, New York

Westendorff, S., B.S., German Primate Center, Goettingen, Germany

SEMINARS

Adelson, T., Massachusetts Institute of Technology, Cambridge: The elements of early vision.

Boynton, G., University of Washington, Seattle: Introduction to Matlab tutorials and fMRI in the LGN and V1.

Brainard, D., University of Pennsylvania, Philadelphia: Color vision.

Carrasco, M., New York University, New York: Attention: Psychophysics and neuroimaging.

Chance, F., University of California, Irvine: Normalization models II.

Chichilnisky, E.J., The Salk Institute, La Jolla, California: Retina I. Retina II.

Dan, Y., University of California, Berkeley: Early ventral stream.

Geisler, W., University of Texas, Austin: Image statistics.

Heeger, D., New York University, New York: Normalization models I.

Hirsch, J., University of Southern California, Los Angeles: LGN/V1 anatomy and physiology.

Horwitz, G., University of Washington, Seattle: V1/white noise analysis.

Movshon, J.A., New York University, New York: MT physiology.

Reid, C., Harvard Medical School, Boston, Massachusetts: V1.

Rust, N., University of Pennsylvania, Philadelphia: Population coding/decoding.

Shadlen, M., University of Washington, Seattle: Decision making.

Simoncelli, E., New York University, New York: Linear systems.

Snyder, L., Washington University School of Medicine, St. Louis, Missouri: Higher dorsal stream.

Sommer, F., Redwood Center for Theoretical Neuroscience, University of California, Berkeley: Models of LGN and V1.

Treue, S., German Primate Center, Goettingen, Germany: Attention physiology.

Tsao, D., California Institute of Technology, Pasadena: Higher ventral stream.

Webster, M., University of Nevada, Reno: High-level adaptation.

Computational Cell Biology

July 2–22

INSTRUCTORS **T. Elston,** University of North Carolina, Chapel Hill
L. Loew, University of Connecticut Health Center, Farmington
G. Smith, College of William & Mary, Williamsburg, Virginia
J. Tyson, Virginia Tech, Blacksburg

ASSISTANTS **S.A. Brown,** University of Connecticut Health Center, Farmington
M. LaMar, College of William and Mary, Williamsburg, Virginia
E. Ramirez Hoyos, University of Connecticut Health Center, Farmington
D. Siegal-Gaskins, Ohio State University, Columbus

Computational cell biology is the field of study that applies the mathematics of dynamical systems together with computer simulation techniques to the study of cellular processes. The field encompasses several topics that have been studied long enough to be well established in their own right, such as calcium signaling, molecular motors and cell motility, the cell cycle, and gene expression during development. In addition to providing a recognizable larger community for topics such as these, this course provided a base for the development of newer areas of inquiry, for example, the dynamics of intracellular second-messenger signaling, of programmed cell death, of mitotic chromosome movements, and of synthetic gene networks. Unlike computational genomics or bioinformatics, computational cell biology focused on simulation of the molecular machinery (genes–proteins–metabolites) that underlie the physiological behavior (input-output characteristics) of living cells.

This 3-week course incorporated a series of didactic lectures on the mathematics of dynamical systems, computational simulation techniques, cell biology, and molecular biology. Practicing theoreticians and experimentalists rotated for 1- to 3-day visits during the course to give lectures and interact with the students. Midway through the course, students selected an area for independent study, and the focus of the last week of the course largely on these projects, supplemented by continued visiting lecturers.

PARTICIPANTS

Alsing, A., M.S., Center for Models of Life, Denmark, Copenhagen

Ay, A., Ph.D., Michigan State University, E. Lansing

Berthoumiel, S., M.Sc., INRIA, Montbonnot, France

Brody, Y., M.Sc., Bar-lian University, Ramat-Gan, Israel

Chettaoui, C., B.A., INRIA and INRA, Paris, France

Clowney, E., B.S., University of California, San Francisco

de Luis B., M.B.A., North Carolina State University, Raleigh

Diedrichs, D., M.S., University of Iowa, Iowa City

Ebisuya, M., Ph.D., Kyoto University, Japan

Gresham, D., Ph.D., New York University, New York

Hoang-Trong, T., B.E., George Mason University, Manassas, Virginia

Jose, D., M.Sc., Max Delbruck Center for Molecular Medicine, Berlin, Germany

Kamenz, J., Dipl., Friedrich Miescher Laboratory, Tubingen, Germany

Keifenheim, D., B.S., University of Massachusetts Medical School, Worcester

Korencic, A., M.A., University of Ljubljana, Slovenia

Long, B., Ph.D., Rice University, Houston, Texas

Marriott, H., Ph.D., University of Sheffield, United Kingdom

Pearce, S., B.S., Purdue University, W. Lafayette, Indiana

Tsui, R., B.A., University of California San Diego

SEMINARS

Blackwell, A., George Mason University Krasnow Institute, Fairfax, Virginia: Signaling pathway in synaptic plasticity and learning. Modeling the role of temporal pattern and spatial localization in synaptic plasticity.

Civelekoglu, S.G., University of California, Davis: Microtubule-based motility in the mitotic spindle and the cilium.

Cytrynbaum, E., University of British Columbia, Vancouver, Canada: Stochastic modeling of polymer dynamics. Stochastic modeling with Matlab.

Hasty, J., University of California, San Diego: Gene networks.

Haugh, J., North Carolina State University, Raleigh: Signaling RTKS.

Hucka, M., California Institute of Technology, Pasadena: SBML + model databases and translation.

Iyengar, R., Mt. Sinai School of Medicine, New York: Signaling models.

Keener, J., University of Utah, Salt Lake City: Models of cardiac physiology. Cardiac exercises.

Loew, L., University of Connecticut Health Center, Farmington: Virtual cell and ODEs. Virtual cell and ODEs excerises. Virtual cell and spatial modeling. Virtual cell and spatial/stochastic exercises. Dendritic nucleation.

Ma'ayan, A., Mount Sinai School of Medicine, New York: Network analysis algorithms and datasets.

Mendes, P., University of Manchester, United Kingdom: Copasi and parameter estimation.

Mogilner, A., University of California, Davis: Microtubule dynamics and centering.

Phair, R., Integrative Bioinformatics, Los Altos, California: Cell biology, metabolism, and tracers.

Sachs, K., Stanford University, California: Bayesian analysis of reaction networks.

Samant, A., Mathworks, Natick, Massachusetts: Network (ode) modeling with SimBiology. Matlab tutorial.

Sherman, A., NIH/National Institute of Diabetes, Digestive, and Kidney Diseases, Bethesda, Maryland: Whole-cell models. Cellular biophysics and ODEs. Reaction-diffusion and advection. Markov chain models in cell biology. Waves.

Terman, D., Ohio State University, Columbus: Dynamical systems. Introduction to XPP/setup and exercises. Dynamical systems and XPP.

Tyson, J., Virginia Tech, Blacksburg: How do cells compute network motifs and functional modules. Temporal organization of the cell cycle. Design principles of biochemical oscillators. XXP exercises. History of computational cell biology XPP exercises.

You, L., Duke University, Durham, North Carolina: Cell cycle. Modeling and engineering bacterial population dynamics by cell–cell communication.

Molecular Techniques in Plant Science

July 2–22

INSTRUCTORS **S. Harmer,** University of California, Davis
R. Last, Michigan State University, E. Lansing
J. Maloof, University of California, Davis

ASSISTANTS **C. Ellison,** University of California, Davis
M. Gaurav, Michigan State University, E. Lansing
A. Schilmiller, Michigan State University, E. Lansing
A. Schrager, University of California, Davis

This course provided an intensive overview of topics in plant physiology, biochemistry, and development, focusing on molecular genetic and analytical approaches to understanding plant biology. It emphasized recent results from *Arabidopsis*, maize, and a variety of other plants and provided an introduction to current methods used in plant molecular biology. It is designed for scientists with some experience in molecular techniques or in plant biology who wish to work with plants using the latest technologies in genetics, molecular biology, and biochemistry. The course consisted of a vigorous lecture series, a hands-on laboratory, and informal discussions. Discussions of important topics in plant research were presented by the instructors and by invited speakers. Seminars included plant morphology and anatomy; plant development (such as development of flowers, leaves, male and female gametophytes, and roots); perception of light and photomorphogenesis; cell-wall biosynthesis, function, and perception of hormones; and application of research results to addressing current agronomic problems. Lectures describing bioinformatics tools available to the plant community, and the resources provided by plant genome projects were also included. Speakers provided overviews of their fields, followed by in-depth discussions of their own work. The laboratory sessions

provided an introduction to important techniques currently used in plant research. These included studies of plant development, mutant analysis, histochemical staining, transient gene expression, gene silencing, applications of fluorescent protein fusions, protein interaction and detection, proteomics approaches, several different approaches for quantifying metabolites, transient transformation, and techniques commonly used in genetic and physical mapping. The course also included several short workshops on important themes in plant research.

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Arias-Garzon, T., B.S., University of Missouri, Columbia

Casas, M., B.S., The Ohio State University, Columbus

Kosma, D., Ph.D., Michigan State University, E. Lansing

Lindh, I., M.Sc., Orebro University, Sweden

Liu, B., M.S., Norwegian University of Science and Technology, Trondheim, Norway

Markelz, R.J.C., B.Sc., Institute of Genomic Biology, Urbana, Illinois

McCarthy Suarez, I., Ph.D., IBMCP-CSIC, Valencia, Spain

Muller, K., Ph.D., Simon Fraser University, Burnaby BC, Canada

Sasidharan, R., Ph.D., Utrecht University, The Netherlands

Sattely, E., Ph.D., Harvard Medical School, Boston, Massachussetts

Schopper, S., M.Sc., Lund University, Sweden

Stephan, A., B.S., The Johns Hopkins University, Baltimore, Maryland

Wilkins, O., B.Sc., Dartmouth College, Hanover, New Hampshire

SEMINARS

Bailey-Serres, J., University of California, Riverside: Plant stress responses.

Birchler, J., University of Missouri, Columbia: Maize genetics.

Birnbaum, K., New York University, New York: Root development.

Bomblies, K., Harvard University, Boston, Massachusetts: Genetics of speciation.

Bowman, J., Monash University, Melbourne, VIC, Australia: Evolution of plant development.

Brutnell, T., Boyce Thompson Institute, Ithaca, New York: Plastids. Short read sequencing.

Coruzzi, G., New York University, New York: Plant systems biology.

Grossniklaus, U., University of Zurich, Switzerland: Gametophyte development.

Harmer, S., University of California, Davis: Circadian rhythms.

He, S.Y., Medical College of Wisconsin, Milwaukee: Plant/pathogen interactions.

Jackson, D., Cold Spring Harbor Laboratory: Shoot meristem development.

Jander, G., Boyce Thompson Institute, Ithaca, New York: Quantitative genetics.

Juntawong, P., University of California, Riverside: Quantitative PCR.

Kellogg, E.T., University of Missouri, St. Louis: Phylogenetics.

Kerk, N., Yale University, New Haven, Connecticut: Introduction to plant biology.

Kuhlemeier, C., University of Bern, Switzerland: Phyllotaxy.

Kutchan, T., Donald Danforth Plant Science Center, St. Louis, Missouri: Bioactive plant metabolites.

Last, R., Michigan State University, E. Lansing: Secondary metabolites.

Maloof, J., University of California, Davis: Light regulation of plant development.

Oldroyd, G., John Innes Centre, Norwich, United Kingdom: Rhizobial interactions/calcium signaling.

Pauly, M., University of California, Berkeley: Cell walls and biofuels.

Poethig, S., University of Pennsylvania, Philadelphia: Vegetative development.

Roossnick, M., University of Colorado, Denver: Plant/virus interactions.

Schaller, G.E., Dartmouth College, Hanover, New Hampshire: Cytokinin signaling.

Shukla, V., Dow Agro Sciences LLC, Indianapolis, Indiana: Modification of plant genome.

Spadling, E., University of Wisconsin, Madison: Photosensory signal transduction.

Sussex, I., Yale University, New Haven, Connecticut: Introduction to plant biology.

Timmermans, M., Cold Spring Harbor Laboratory: microRNA regulation.

Vision, T., University of North Carolina, Durham: Genome evolution/speciation.

Walling, L., University of California, Riverside: Plant/pest interactions.

Zheng, N., University of Washington, Seattle: Regulated protein degradation and plant.

Neurobiology of *Drosophila*

July 2–22

INSTRUCTORS   **R. Allada,** Northwestern University, Evanston, Illinois
H. Broihier, Case Western Reserve University, Cleveland, Ohio
D. Featherstone, University of Illinois, Chicago

ASSISTANTS   **T. Andlauer,** FU Berlin, Germany
K. Chen, University of Illinois, Chicago
V. Kilman, Northwestern University, Evanston, Illinois
C. Miller, Case Western Reserve University, Cleveland, Ohio
I. Nechipurenko, Case Western Reserve University, Cleveland, Ohio
D. Owald, Charite Berlin, Germany

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students through established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The 3-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that are used in the investigation of current neurobiological questions. The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization, labeling of identified neurons, electrophysiology (intracellular recording and patch clamping), optical imaging of neuronal activity, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system.

PARTICIPANTS

Aw, S., Ph.D., Duke–NUS Graduate Medical School, Singapore

Bahl, A., Dipl., Max-Planck Institute of Neurobiology, Martinsried, Germany

Duncan, K., Ph.D., EMBL, Heidelberg, Germany

Gruber, M., M.A., Johannes Gutenberg-University Mainz, Germany

Halstead, J., M.Sc., Oxford University, United Kingdom

Keller, P., Dipl., HHMI/Janelia Farm Research Campus, Ashburn, Virginia

Lee, L., B.S., Florida Atlantic University, Boca Raton

Ramdya, P., Ph.D., UNIL/EPFL, Lausanne, Switzerland

Robertson, D., Sc.B., University of California, San Francisco

Schweizer Burguete, A., Ph.D., University of Pennsylvania, Philadelphia

Tschida, K., B.A., Duke University, Durham, North Carolina

Vanlandingham, P., Ph.D., University of Oklahoma, Norman

SEMINARS

Allada, R., Northwestern University, Evanston, Illinois: Circadian rhythms and sleep.

Benton, R., University of Lausanne, Switzerland: Olfactory signal processing and physiology/imaging of the olfactory system.

Broihier, H., Case Western University, Cleveland, Ohio: Axon Pathfinding. Genetic screens.

Dubnau, J., Cold Spring Harbor Laboratory: Learning and memory.

Ejima, A., Kyoto University, Japan: Courtship.

Featherstone, D., University of Illinois, Chicago: Information transfer in the nervous system and introduction to electrophysiology.

Fouquet, W. and Andlauer, T.F., Freie Universitat Berlin, Germany: Synpatic ultrastructure and live imaging of fluorescently-tagged synaptic proteins.

Freeman, M., University of Massachusetts, Worcester: Glia. Bioinformatics/web tools.

Goodwin, S., Glasgow University, United Kingdom; Jefferis, G., University of Cambridge, United Kingdom: Mosaic techniques. Adult brain anatomy.

Lee, C., NIH/National Institute of Child Health and Human Development, Bethesda, Maryland: Visual system development.

Levitan, E., University of Pittsburgh, Pennsylvania: Imaging, peptide release/dense core vesicle trafficking.

Luo, L., Stanford University, California: Combined lecture with advanced techniques in molecular neurobiology course.

O'Connor-Giles, K., University of Wisconsin, Madison: NMJ structure and development.

Rothenfluh, A., University of Texas Southwestern, Dallas: Ethanol/drugs.

Shen, P., University of Georgia, Athens: Larval feeding behavior.

Skeath, J., Washington University School of Medicine, St. Louis, Missouri: Neural stem cells.

Turner, G., Cold Spring Harbor Laboratory: CNS physiology and imaging.

Verstreken, P., K.U. Leuven, Belgium: Vesicle cycling and neurotransmitter release.

Waddell, S., University of Massachusetts, Worcester: Learning and memory.

Genetics of Complex Human Diseases

July 6–12

INSTRUCTORS   **A. Al-Chalabi,** Kings College London, United Kingdom
                             L. Almasy, Southwest Foundation for Biomedical Research, San Antonio,

Complex diseases are conditions that are influenced by the actions of multiple genes, their interactions with each other and with the environment. This lecture course considered the difficulties in 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, including family and case/control studies, as well as methods for quantifying the strength of the genetic influences on a disease. A major focus was the identification of specific gene effects using both linkage and association analysis and their variants. We discussed the efficiency and robustness of different designs for such analysis and 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 was covered as well as copy-number variation, haplotype mapping, SNP tagging, meta-analysis, and gene–environment interaction. An overview of next-generation sequencing 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.

PARTICIPANTS

Brisbin, A., B.S., Cornell University, Ithaca, New York

Byrne, S., Ph.D., Trinity College, Dublin, Dundalk, Ireland

Cropp, C., Ph.D., National Human Genome Research Institute/NIH, Baltimore, Maryland

Demacq, C., Ph.D., State University of Campinas (UNICAMP), Campinas, Brazil

Dunn, E., B.S., Harvard School of Public Health, Norwood, Massachusetts

Hall, D., Ph.D., Rush University, Chicago, Illinois

Jiang, Y., B.S., Emory University, Atlanta, Georgia

Kepp, K., B.S., Estonian Biocentre and University of Tartu, Estonia

Li, R., Ph.D., National Institutes of Health, Rockville, Maryland

Losh, M., Ph.D., University of North Carolina, Chapel Hill

Manjarrez Orduño, N., Ph.D., The Feinstein Institute, Manhasset, New York

McLaughlin, R., B.S., Trinity College, Dublin, Ireland

Melton, P., Ph.D., Southwest Foundation for Biomedical Research, San Antonio, Texas

Moghraby, J., Ph.D., Kind Saud bin Abdul Aziz University-Health Science, Riyadh, Saudi Arabia

Nielsen, K., B.S., Technical University of Denmark, Lyngby

Polsky, D., Ph.D., New York University Langone Medical Center, New York

Reynolds, R., Ph.D., University of Alabama, Birmingham

Shulman, J., Ph.D., Brigham & Women's Hospital, Boston, Massachusetts

Tiwari, A., Ph.D., Center for Addiction & Mental Health, Toronto, Canada

Wang, Y., B.S., Center for Integrative Genetics; Aas, Norway

Zhang, J., Ph.D., The Zucker Hillside Hospital, Glen Oaks, New York

Zlojutro, M., Ph.D., Southwest Foundation for Biomedical Research, San Antonio, Texas

SEMINARS

Al-Chalabi, A., King's College London, United Kingdom: GWAS and principles of association.

Almasy, A., Southwest Foundation for Biomedical Research, San Antonio, Texas: Introduction to linkage and quantitative traits.

Borecki, I., Washington University School of Medicine, St. Louis, Missouri: Genetic epidemiology.

de Bakker, P., Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts: GWAS–Meta-analysis.

Loos, R., Cambridge University, United Kingdom: GxE interaction.

Neale, B., Massachusetts General Hospital, Boston: SNP imputation.

Purcell, S., Massachusetts General Hospital, Boston: Analysis of GWAS.

Rampersaud, E., University of Miami, Florida: Family-based association.

Sham, P.-C., University of Hong Kong, China: Power considerations.

Sinsheimer, J., David Geffen School of Medicine, Los Angeles, California: Statistics 101.

Tobin, M., University of Leicester, United Kingdom: Copy-number variation.

Trembath, R., King's College London, United Kingdom: Next-generation sequencing

Advanced Techniques in Molecular Neuroscience

July 6–22

INSTRUCTORS **C. Lai,** Indiana University, Bloomington
R. Lansford, California Institute of Technology, Pasadena
B. Stevens, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts

ASSISTANTS **S. Gattu,** Indiana University, Bloomington
D. Huss, California Institute of Technology, Pasadena
E. Lehrman, Harvard University/Children's Hospital, Boston, Massachusetts
K. Podorski, University of British Columbia, Vancouver, Canada
E. Robinson, Indiana University, Bloomington
A. Rosen, Harvard Medical School, Boston, Massachusetts

This course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the advanced techniques in molecular neuroscience. The course was geared toward students who were familiar with the basic techniques of molecular biology.

The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises, daily lectures on theoretical and practical aspects of advanced molecular biological and imaging techniques, and a series of evening lectures given by invited speakers which served to showcase the ways in which these molecular techniques have been used to advance specific areas of neurobiology. The laboratory exercises were designed to provide each student with a working familiarity with these advanced techniques using proven protocols and reagents. The experiments and protocols were chosen to provide the course participants with sufficient hands-on expertise so that they may return to their home laboratories and, with a modicum of practice and perseverance, successfully implement the use of these advanced technologies.

Each laboratory exercise was preceded by a lecture that covered the purpose of the experiment and included an in-depth presentation of the concepts, background, and theory. The participants were guided through a step-by-step analysis of the actual protocol, which were provided both in hard copy and on the course website. The laboratory portion of each course included gene delivery systems including mammalian cell transfection, single-cell electroporation, the selection and use of animal viral vectors, as well as the introduction of DNAs using in vivo electroporation in *Xenopus* tadpoles, quail embryos, and rodents; loss-of-function approaches to study gene function using RNAi approaches; the use of optogenetics to probe neural circuits within animals; the design and manipulation of bacterial artificial chromosome (BAC) transgenic vectors and their use in achieving cell-type-specific expression in animals; comparison of quantitative PCR, RNA-Seq, and microarray analyses to profile gene expression; methods to identify gene expression in single cells and in sub-cellular regions of single cells; and techniques to isolate, propogate, and analyze desired neural and glial populations. As many of these advanced techniques also included the use of more standard procedures to first modify and then "move" DNA fragments from one vector to another, the laboratory exercises also provided training in these methods, which were essential to the successful execution of the more sophisticated approaches. Finally, course participants were introduced to bioinformatics and a wide range of Internet resources that are available to molecular neuroscientists.

PARTICIPANTS

Bajic, D., Ph.D., Children's Hospital, Boston, Massachusetts

Barrett, R., B.A., University of California, Irvine

Brouwers, N., Ph.D., VIB and University of Antwerp, Belgium

Hayden, M., M.A., Stanford University Hospital and Clinics, California

He, H., Ph.D., The Scripps Research Institute, La Jolla, California

Heng, M., B.S., Vollum Institute, OHSU, Portland, Oregon

Heraud, J., B.Sci., Medical University of Vienna, Austria

Kirby, E., B.S., University of California, Berkeley

Klaus, A., M.Sc., National Institutes of Health, Bethesda, Maryland

Lotfipour, S., Ph.D., University of California, Los Angeles

Nogaroli, L., Ph.D., Federal University of Rio de Janeiro, Brazil

Sink, K., Ph.D., Emory University, Atlanta, Georgia

Stephenson-Jor, M., B.Sc., Karolinska Institutet, Stockholm, Sweden

Toledo, A., M.S., Universidad de la Republica, Monvevideo, Uruguay

Wheeler, T., B.S., University of Rochester, New York

Zong, C., Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Barres, B., Stanford University School of Medicine, California: Neuron–glial interactions in the CNS.

Cline, H., The Scripps Research Institute, La Jolla, California: Distinguished Alumni Lecture. Molecular dissection of neural circuit development.

Darnell, R., The Rockefeller University, New York: Decoding RNA maps in the brain.

Eberwine, J., University of Pennsylvania Medical School, Philadelphia: Metastability of the henome: Impact on neuronal phenotype and dendritic biology.

Lichtman, J., Harvard University, Cambridge, Massachusetts: Connectomics.

Lipscombe, D., Brown University, Providence, Rhode Island: Neuronal calcium channels: Spliced for optimal function.

Liquin, L., Stanford University, California: Mapping olfaction in the fly and mouse brain.

Lledo, P.M., Pasteur Institute, Paris, France: Development of neural stem cells in the adult circuits.

Workshop on Biology of Social Cognition

July 14–20

INSTRUCTORS   **J. Mitchell,** Harvard University, Cambridge, Massachusetts
                           D. Skuse, University College London Institute of Child Heath, United Kingdom

ASSISTANT   **D. Tamir,** Harvard University, Cambridge, Massachusetts

The past few years have seen remarkable advances in our knowledge of the genetic, molecular, and neural factors that contribute to social behavior. At the same time, sophisticated analytical and theoretical approaches have helped to make sense of the data. This week-long workshop sought to provide a comprehensive overview of these topics. Although the emphasis was on social cognition in humans, there were also study days dedicated to state-of-the-art presentations on comparative approaches and evolutionary models. Finally, all themes were related to the clinical consequences of dysfunctional social cognition and the role of translational research. The course included introductory seminars on key themes, offered every morning. There were afternoons devoted largely to practical sessions that provided hands-on experiments in consultation with seminar leaders, rounded off by after-supper keynote lectures by leading scientists that reflect cutting-edge and future views related to our theme for that day.

This workshop was supported with funds provided by Autism Speaks; the INCORE Consortium; Nancy Lurie Marks Family Foundation; and the Simons Foundation.

PARTICIPANTS

Albo, Z., B.S., Baylor College of Medicine, Houston, Texas

Bartholomeusz, C., Ph.D., The University of Melbourne, Carton South, Australia

Chevallier, C., Ph.D., King's College London, United Kingdom

Contreras, J., B.S., Harvard University, Cambridge, Massachusetts

Cook, J., B.S., University College London, United Kingdom

Costa, R., B.S., University of Lisbon, Portugal, Lisboa

Diehl, M., B.S., University of Rochester, New York

Hillebrandt, H., B.S., University College London, United Kingdom

Koelkebeck, K., Ph.D., University of Muenster, Germany

Kret, M., B.S., Tilburg University, The Netherlands

Lee, V., B.S., McMaster University, Hamilton, Canada

Light, S., B.S., University of Wisconsin, Madison

Menyhart, O., B.S., Cornell University, Ithaca, New York

Moore, L., B.S., University of California, Los Angeles

Moore III, W., B.S., University of Oregon, Eugene

Muralidharan, A., B.S., Emory University, Atlanta, Georgia

Paxton, R., B.S., Emory University, Atlanta, Georgia

Simmons, J., Ph.D., National Institutes of Health, Bethesda, Maryland

Stjepanovic, D., B.S., The University of Queensland, St. Lucia, Australia

Theodoridis, A., B.S., University of California, Berkeley

Troiani, V., B.S., University of Pennsylvania School of Medicine, Philadelphia

Zaki, J., B.S., Columbia University, New York

SEMINARS

Amodio, A., New York University, New York: Social cognition and emotion.

Blair, J., National Institute of Mental Health, Bethesda, Maryland: Psychopathy.

Blakemore, S., University College of London, United Kingdom: Development of social cognition.

Bourgeron, T., Pasteur Institute, Paris, France: Autism genetics.

Cahill, L., University of California, Irvine: Sex differences in emotional processing.

Couzin, I., University of Princeton, New Jersey: Evolution of collective behavior.

Cushman, F., Harvard University, Cambridge, Massahusetts: The moral faculty: Domain specificity, neural circuitry, and universality.

Dean, M., Brown University, Providence, Rhode Island: Introduction to neuroeconomics.

Giedd, J., National Institute of Mental Health, Bethesda, Maryland: Neuroimaging of the developing brain.

Haxby, J., Dartmouth College, Hanover, New Hampshire: Face processing.

Huhman, K., Georgia State University, Atlanta: Conditioned defeat in hamsters.

Matsuzawa, T., Kyoto University, Aichi, Japan: Social cognition in primates.

Mitchell, J., Harvard University, Cambridge, Massachusetts: Investigating theory of mind.

Murray, R., King's College London, United Kingdom: Social cognition and major mental illness.

Ochsner, K., Columbia University, New York: Regulation of emotion and social cognition.

Phelps, E., New York University, New York: Emotion and cognition.

Schultz, B., Children's Hospital of Philadelphia, Pennsylvania: The neural basis of autism.

Shaw, P., National Institutes of Mental Health, Bethesda, Maryland: Genes, brain, and behavior.

Skuse, D., University College London, United Kingdom: The biology of social cognition in autistic disorders. X-linked genes and social cognition.

Wieben, E., Mayo Clinic, Rochester, Minnesota: Introduction to genomics.

Young, L., Emory University, Atlanta, Georgia: Neuropeptides and social cognition: From pair-bonding voles to autism.

Brain Tumors

July 22–28

INSTRUCTORS   **D. Gutmann,** Washington University School of Medicine, St. Louis, Missouri
                             E. Holland, Memorial Sloan-Kettering Cancer Center, New York
                             S. Majumder, University of Texas M.D. Anderson Cancer Center, Houston

ASSISTANT   **S. Singh,** University of Texas M.D. Anderson Cancer Center, Houston

This one-week discussion course provided a clinical overview of brain tumors and emphasized molecular mechanisms involved in the growth and development of brain tumors with special emphasis on neural differentiation, signaling mechanisms, DNA replication, chromatin modulation, stem cells, mouse models, genomics, imaging techniques, genetically modified mouse techniques, nanotechnology, mechanism-based therapeutic strategies, biobanks, and ethical concerns. Attendees were able to interact with senior investigators on a one-to-one basis in an informal environment.

PARTICIPANTS

Annibali, D., Ph.D., CNR, Toronto, Canada

Aref, D., B.S., University of Toronto, Canada

Dziurzynski, K., Ph.D., University of Texas M.D. Anderson Cancer Center, Houston

Gajadhar, A., B.S., Hospital for Sick Children, University of Toronto, Canada

Jones, T., Ph.D., University of Tennessee, Memphis

Kohli, L., B.S., University of Alabama, Birmingham

Kristoffersen, K., B.S., Copenhagen National University Hospital, Denmark

Lathia, J., Ph.D., Cleveland Clinic Foundation, Ohio

Li, S., Ph.D., University of California, Los Angeles

McDevitt, M., Ph.D., Memorial Sloan-Kettering Cancer Center, New York

Moiyadi, A., Ph.D., Tata Memorial Center, Mumbai, India

Panosyan, E., Ph.D., University of California, Los Angeles

Paolella, B., B.S., Dartmouth College, Lebanon, New Hampshire

Põlajeva, J., B.S., Uppsala University, Sweden

Pollard, S., Ph.D., University College London, United Kingdom

Read, R., Ph.D., The Salk Institute for Biological Studies, La Jolla, California

Rich, B., Ph.D., Dana Farber Cancer Institute, Boston, Massachusetts

Sathyan, P., Ph.D., M.D. Anderson Cancer Research Center, Houston, Texas

Song, Y., Ph.D., National Cancer Institute, Frederick, Maryland

Vanner, R., B.S., University of Toronto, Canada

Wells, E., Ph.D., Children's National Medical Center, Washington, D.C.

Zinn, P., Ph.D., Dana Farber Cancer Institute/Harvard University, Boston, Massachusetts

SEMINARS

Aldape, K., University of Texas M.D. Anderson Cancer Center, Houston: Molecular subgroups of gliomas and prognostic markers.

Becher, O., Memorial Sloan-Kettering Cancer Center, New York: Clinical aspects of pediatric brain tumors.

Bondy, M., M.D. Cancer Center, Houston, Texas: Genetic and molecular epidemiology of brain tumors.

Brennan, C., Memorial Sloan-Kettering Cancer Center, New York: Molecular subclasses of glioma and accessing The Cancer Genome Atlas.

Eberhart, C., Johns Hopkins University School of Medicine, Baltimore, Maryland: The role of Notch signaling in brain tumor initiation and growth.

Enikolopov, G., Cold Spring Harbor Laboratory: Quiescence and division of neural stem cells.

Fuller, G., M.D. Anderson Cancer Center, Houston, Texas: Clinical overview of brain tumors: Neuroradiology and neuropathology.

Gilbertson, R., St. Jude's Children's Research Hospital, Memphis, Tennessee: Developmental neurobiology oncology.

Gladson, C., Cleveland Clinic, Ohio: Angiogenesis in malignant glioma tumors: Can we effectively target it?

Gutmann, D., Washington University School of Medicine, St. Louis, Missouri: Using neurofibromatosis-1 to understand pediatric brain tumors.

Holland, E., Memorial Sloan-Kettering Cancer Center, New York: Modeling proneural/PDGF gliomas in mice.

James, C.D., University of California, San Francisco: Transplantable orthotopic tumor models for therapeutic testing.

Kornblum, H., University of California Molecular and Medical Pharmacology, Los Angeles: Methods and rationales in evaluating neural and brain tumor stem cells.

Lang, Jr., F., M.D. Anderson Cancer Center, Houston, Texas: Clinical management of brain tumors: The surgical perspective.

Majumder, S., University of Texas M.D. Anderson Cancer Center, Houston: Cellular flexibility determines both normal brain development and brain tumors.

Pelroy, R., National Cancer Institute, Bethesda, Maryland: Funding opportunities at the National Institutes of Health.

Pieper, R., University of California, San Francisco: The multiple roles of ubiquitination in the development and treatment of brain cancer.

Reilly, K., National Cancer Institute, Bethesda, Maryland: Using mouse models of brain cancer to understand genetic susceptibility factors.

Rich, J., Cleveland Clinic, Ohio: Brain tumor stem cells.

Roussel, M., St. Jude's Children's Research Hospital, Memphis, Tennessee: Critical signaling pathways in cerebellum development and cancer.

Rowitch, D., University of California, San Francisco: Generation of mouse models of pediatric brain cancer.

Snyder, E., Burnham Institute, La Jolla, California: Using our growing knowledge of fundamental stem cell biology to understand brain tumor development and behavior.

Stiles, C., Dana Farber Cancer Institute, Boston, Massachusetts: Separated at birth? Transcription factors in normal and malignant neural progenitors.

Wechsler-Reya, R., Duke University Medical Center, Durham, North Carolina: What mouse models teach us about medulloblastoma biology.

Yung, W.K.A., M.D. Anderson Cancer Center, Houston, Texas: Molecular targeted therapy in malignant gliomas.

Proteomics

INSTRUCTORS   **T. Andacht,** Centers for Disease Control and Prevention, Atlanta, Georgia
J. Bruce, University of Washington, Seattle
A. Link, Vanderbilt University School of Medicine, Nashville, Tennessee
D. Pappin, Cold Spring Harbor Laboratory

ASSISTANTS   **K. Bayyareddy,** University of Georgia, Athens
C. Browne, Vanderbilt University School of Medicine, Nashville, Tennessee
C. Fu, Cold Spring Harbor Laboratory
P. Kirby, University of Georgia, Athens
S. Peacock, Cold Spring Harbor Laboratory
C. Ruse, Cold Spring Harbor Laboratory
P. Samir, Vanderbilt University School of Medicine, Nashville, Tennessee
B. Weindorf, Cold Spring Harbor Laboratory
C. Weisbrod, University of Washington, Seattle

This intensive laboratory and lecture course focused on cutting-edge proteomics approaches and technologies. In the protein-profiling portion of the course, students gained hands-on experience in several quantitative proteome analysis methods, including two-dimensional gel electrophoresis and isotopic labeling strategies. Students were trained to use DIGE (differential in-gel electrophoresis) for gel-based protein quantification. Differentially expressed proteins were determined using advanced gel analysis software and identified using MALDI mass spectrometry. Students were taught

differential and quantitative mass spectrometry approaches to profile and identify changes in proteomes and were trained in high-sensitivity microcapillary liquid chromatography coupled with nanospray-ESI and tandem mass spectrometry. Both single dimension and multidimensional microcapillary liquid chromatography separations coupled to mass spectrometry were taught. Students gained hands-on experience purifying and identifying protein complexes and posttranslational modifications using the latest technologies. This course also included discussion and application of multiple reaction monitoring (MRM) methods for targeted proteomics. Students developed and applied MRM transitions and chromatographic methods to quantify targeted proteins in complex proteome samples, such as serum or whole-cell lysates. A strong emphasis was placed on data analysis throughout the course. The overall aim of the course was to provide students with the fundamental knowledge and hands-on experience necessary to be able to perform and analyze proteomics experiments and to learn to identify new opportunities in applying proteomics approaches to their own research.

This course was supported with funds provided by the National Cancer Institute and the Howard Hughes Medical Institute.

PARTICIPANTS

Bellail, A., Ph.D., Emory University, Atlanta, Georgia
Cilia, M., Ph.D., USDA-ARS, Ithaca, New York
Gruninger, J., M.Sc., University of British Columbia, Vancouver, Canada
Igel, U., M.A., Royal Institute of Technology, Stockholm, Sweden
Kigar, S., B.A., University of Wisconsin, Madison
Kimura, M., Ph.D., Gifu University School of Medicine, Japan
Leal, M., B.A., Federal University of Sao Paulo, Brazil
Mande, K., Ph.D., King Abdullah University of Science and Technology, Jeddah, Saudi Arabia
McDonald, Z., B.Sc., University of Cape Town, South Africa

Møldrup, M., M.S., University of Copenhagen, Denmark
Moore, R., Ph.D., National Institute of Allergy & Infectious Disease, Hamilton, Montana
Richlen, M., Ph.D., Woods Hole Oceanographic Institution, Massachusetts
Roedkaer, S., B.A., University of Southern Denmark, Odense M, Denmark
Seizl, M., Ph.D., Ludwig-Maximilians-Universistät, Munich, Germany
Zarr, M., B.A., Johns Hopkins School of Medicine, Baltimore, Maryland
Zou, J., B.S., Xavier University of Louisiana, New Orleans

SEMINARS

Andacht, T., Centers for Disease Control and Prevention, Atlanta, Georgia: Fundamentals and applications of two-dimensional DIGE in proteomics.
Bruce, J., University of Washington, Seattle: Fundamentals of mass spectrometry and proteomics.
Chait, B., The Rockefeller University, New York: Proteomic analysis of protein machines.
Chaurand, P., University of Montreal, Canada: Imaging mass spectrometry of cells and tissue.
Clauser, K., Broad Institute of MIT and Harvard, Cambridge, Massachusetts: De novo interpretation of tandem mass spectra.
Cristea, I., Princeton University, New Jersey: Proteomic probes of the living interactome.
Dufresne, C., Thermo Fisher Scientific, West Palm Beach, Florida: Mass spectrometry approaches to identify and quantify PTMs.
Eng, J., University of Washington, Seattle: Analysis of tandem MS data using the transproteomic pipeline.

Friedman, D., Vanderbilt University, Nashville, Tennessee: Variation and experimental design in proteomic analyses.
Kelleher, N., Northwestern University, Evanston, Illinois: Top-down mass spectrometry.
MacCoss, M.J., University of Washington, Seattle: Targeted proteomics for quantitative analysis and biomarker validation.
Magee, M., Arizona State University, Tempe: Functional proteomics: Protein microarrays.
Pappin, D., Cold Spring Harbor Laboratory: Quantitative proteomics and iTRAQ.
Wolf-Yadlin, A., Harvard University, Cambridge, Massachusetts: Enhanced phosphoproteomics in mammalian systems.
Zhang, B., Vanderbilt University School of Medicine, Nashville, Tennessee: Functional and network analysis of proteomics data.

Eukaryotic Gene Expression

INSTRUCTORS   **J. Espinosa,** HHMI/University of Colorado, Boulder
W.L. Kraus, Cornell University, Ithaca, New York
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri
D. Taatjes, University of Colorado, Boulder

ASSISTANTS   **M. Galbraith,** University of Colorado, Boulder
M. Knuesel, University of Colorado, Boulder
M. Mohan, Stowers Institute for Medical Research, Kansas City, Missouri
D. Ruhl, Cornell University, Ithaca, New York

This course was designed for students, postdocs, and principal investigators who have recently ventured into the exciting area of gene regulation. The course focused on state-of-the-art strategies and techniques used in the field. Emphasis was placed on both in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. Characterizations of the DNA-binding properties of site-specific transcription factors was carried out using electrophoretic mobility shift and DNase I footprinting assays. In addition, students learned techniques for the assembly and analysis of chromatin in vitro. This included transcription assays, chromatin footprinting, and chromatin remodeling assays. During the past few years, the gene regulation field has developed in vivo approaches to study gene regulation. Students were exposed to the chromatin immunoprecipitation technique. They also used RNAi for specific knock-down experiments in mammalian cells. In addition, determining cellular gene expression profiles has been accelerated tremendously by DNA microarray technology. Students received hands-on training in performing and interpreting results from DNA microarrays.

PARTICIPANTS

Bistulfi, G., B.S., Roswell Park Cancer Institute, Buffalo, New York

Camp, J., B.S., University of North Carolina, Chapel Hill

Chatterjee, D., M.Sc., Cold Spring Harbor Laboratory

Chen, S., Ph.D., University of California, San Francisco

de Oliveira, K., B.S., Max Delbrück Center, Berlin, Germany

Duncan, A., M.S., University of Texas, Houston

Gehani, S., M.S., Biotech Research and Innovation Centre, Copenhagen N, Denmark

Hoeg, F., Dipl., Gene Center at the University of Munich, Germany

Klein, A., Ph.D., University of Texas Southwestern Medical Center, Dallas

Kostromitskaia, J., M.S., Centre for Molecular Biology and Neuroscience, Oslo, Norway

Mazumdar, S., M.S., Tufts University, Boston, Massachusetts

Migliori, V., M.D., Institute of Molecular and Cellular Biology, Singapore

Nichol, J., Ph.D., McGill University, Montreal, Canada

Sharma, N., M.S., University of Aarhus, Denmark

Treutlein, B., M.S., Ludwig-Maximilians University, Munich, Germany

Zhang, X., Ph.D., Cornell University, Ithaca, New York

SEMINARS

Adelman, K., National Institute of Environmental Health Sciences/NIH, Research Triangle Park, North Carolina: Pausing of RNA polymerase II disrupts DNA-encoded nucleosome organization to enable precise gene regulation.

Almouzni, G., Institut Curie, Paris, France: Variation on the theme of chromatin assembly.

Carey, M., University of California School of Medicine, Los Angeles: Biochemical mechanisms of chromatin transcription.

Conaway, J., Stowers Institute for Medical Research, Kansas City, Missouri: Introduction to the general transcription machinery.

Cramer, P., LMU University of Munich, Germany: Structure-function studies of eukaryotic transcription.

Gill, G., Tufts University School of Medicine, Boston, Massachusetts: Regulation of chromatin structure and transcription by Sp3 and SUMO.

Kadonaga, J., University of California, San Diego, La Jolla: Secrets of the RNA polymerase II core promoter.

Kingston, R., Massachusetts General Hospital/Harvard Medical School, Boston: In vivo biochemistry: Interrogating nucleosomes and nucleosome-modifying complexes on the genome.

Kurdistani, S., University of California, Los Angeles: Role of a linker histone in regulating expression of gene family clusters.

Levine, M., University of California, Berkeley: Whole-genome analysis of transcriptional precision in the *Drosophila* embryo.

Lieb, J., University of North Carolina, Chapel Hill: Genome-wide measurement of transcription-factor-binding dynamics by competition ChIP.

Luger, K., HHMI/Colorado State University, Fort Collins: The expected, the unexpected, and the just plain weird: Insights from new experimental approaches to study nucleosome structure.

Pugh, F., Pennsylvania State University, University Park: Genome-wide interplay of the transcription machinery and chromatin.

Spector, D., Cold Spring Harbor Laboratory: Intranuclear dynamics.

Washburn, M., Stowers Institute for Medical Research, Kansas City, Missouri: Driving biological discovery with quantitative shotgun proteomics.

Imaging Structure and Function in the Nervous System

July 27–August 16

INSTRUCTORS   **S. Thompson,** University of Maryland School of Medicine, Baltimore
                     W. Tyler, Arizona State University, Tempe
                     J. Waters, Northwestern University, Chicago, Illinois

ASSISTANTS   **O. KoHo,** University College London, United Kingdom
                     A. Nimmerjahn, Stanford University, California
                     S. Page, University of Maryland School of Medicine, Baltimore
                     A. Scimemi, National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland

Advances in light microscopy, digital image processing, and the development of a variety of powerful fluorescent probes present expanding opportunities for investigating the nervous system, from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies. The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, functional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated (caged) compounds, exocytosis tracers, and optogenetic activators. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multiphoton laser-

scanning microscopy and to newly available biological fluorophores, especially green-fluorescent protein (GFP) and its variants. We used a spectrum of neural and cell biological systems, including living animals, brain slices, and cultured cells.

This course was supported with funds provided by the National Institute of Mental Health and the Howard Hughes Medical Institute.

PARTICIPANTS

Clancy, K., B.S., University of Berkeley, California
Cohen, L., Ph.D., The Hebrew University of Jerusalem, Israel
Ford, C., Ph.D., Oregon Health & Science University, Portland
Gu, Y.I., B.S., Johns Hopkins University, Baltimore, Maryland
Kreile, A., M.S., Max-Planck Institute of Neurobiology, Martinsried, Germany
Nadif, K.N., Ph.D., CSHL/DCN, Radboud University, Cold Spring Harbor

Russo, M., B.A., Columbia University, New York
Shobe, J., Ph.D., University of California, Los Angeles
Shuai, Y., Ph.D., Cold Spring Harbor Laboratory
Tremblay, M.-E., Ph.D., University of Rochester Medical Center, New York
van Ham, T., Ph.D., Massachusetts General Hospital/Harvard Medical School, Boston
Xu, S. (Pei), B.S., Washington University, St. Louis, Missouri

SEMINARS

Albeanu, F., Cold Spring Harbor Laboratory: Synapto-phlourins and synaptic vesicle imaging,
Bruchez, M., Carnegie Mellon University, Pittsburgh, Pennsylvania: New and future indicators.
Deisseroth, K., Stanford University, California: Advanced optogenetics/optical probing of brain circuits.
Denk, W., Max-Planck Institute for Medical Research, Heidelberg, Germany: Extended 2P imaging and block-face EM.
DiGregorio, D., Universite Paris 5, France: Flash photolysis.
Emilani, V., CNRS, INSERM, University Paris Descartes, France: Holography.
Engert, F., Harvard University, Cambridge, Massachusetts: Shot noise. 2P microscopy.
George, N., Olympus America, Inc., Center Valley, Pennsylvania: Objectives. BX51 optics.
Gould, T., Yale University School of Medicine, New Haven, Connecticut: STED and PALM.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Bayern, Germany: XFPs and genetically encoded sensors.
Hirsch, J., Columbia University, New York: fMRI.
Huebener, M., Max-Planck Institute of Neurobiology, Martinsried, Germany: Intrinsic imaging.
Kilborn, K., Intelligent Imaging Innovations, Inc., Santa Monica, California: Deconvolution.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Basic microscopy.
Lee, J., University of California, Los Angeles: Advanced optogenetics.

Litchman, J., Harvard University, Cambridge, Masschusetts: Confocal microscopy.
Mrsic-Flogel, T., University College London, United Kingdom: Organic calcium indicators bulk loading.
Prakriya, M., Northwestern University, Evanston, Illinois: Foerster resonance energy transfer.
Racca, C., Newcastle University, Newcastle upon Tyne, United Kingdom: Electron microscopy.
Simpson, J., MBF Bioscience, San Diego, California: MBF, sterology, and structure analysis.
Stutzmann, G., Rosalind Franklin University, N. Chicago, Illinois: Two-photon imaging in AD mouse models.
Tsai, P., University of California, San Diego, La Jolla: Optics bench lab exercises–Basics Koehler. Optics bench lab exercises–Scanning. Scanning and fluorescence. Optics bench lab exercises–Scanning. Optics bench lab exercises–Confocal. CCD cameras.
Tyler, J., Arizona State University, Tempe: Image J and Fiji; Ultrasound neuromodulation.
Waters, J., Northwestern University, Chicago, Illinois: Organic calcium indicators. Single-cell Ca imaging in vivo. CCD cameras; Homebrew microscope including laser safety.
Wilbrecht, L., University of California, San Francisco: GFP applications spine morphology.
Witkowski, J., Cold Spring Harbor Laboratory: Ethics.
Zhang, F., Harvard University, Cambridge, Massachusetts: Light-triggered activation/silencing.

Yeast Genetics and Genomics

July 27–August 16

INSTRUCTORS
D. Burke, University of Virginia, Charlottesville
B. Errede, University of North Carolina, Chapel Hill
J. Smith, University of Virginia, Charlottesville

ASSISTANTS
K. Gardner, University of North Carolina, Chapel Hill
J. McClure, University of Virginia, Charlottesville
M. Wells, University of Virginia, Charlottesville

This is a modern, state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches are emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques, including various types of yeast transformation, gene replacement with plasmids and PCR, construction and analysis of gene fusions, and generation of mutations in cloned genes, were also emphasized. Students used classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality. Students were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. Students gained first-hand experience in modern cytological approaches such as epitope tagging and imaging yeast cells using indirect immunofluorescence, GFP–protein fusions,

and a variety of fluorescent indicators for various subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

PARTICIPANTS

Abeydeera, N.D., Ph.D., Texas A&M University, College Station
Bohnert, M., Ph.D., University of Freiburg, Germany
Bosman, J., Ph.D., University of Groningen, Haren, The Netherlands
Bussemaker, H., Ph.D., Columbia University, New York
Chakravarty, A., M.Sc., Memorial Sloan-Kettering Cancer Center, New York
Chang, M., Ph.D., Nanyang Technological University, Singapore
De Haro, L., Ph.D., University of New Mexico, Albuquerque

Elfick, A., Ph.D., University of Edinburgh, United Kingdom
Goltermann, L., M.Sc., Copenhagen University, Denmark
Hofree, M., B.Sc., University of California, San Diego, La Jolla
Houser, J., B.S., University of North Carolina, Chapel Hill
Kogej, T., Ph.D., University of Ljubljana, Slovenia
Rosby, R., Ph.D., Brown University, Providence, Rhode Island
Srivas, R., Ph.D., University of California, San Diego, La Jolla
Stein, V., MS, University of Queensland, Brisbane, Australia
Waisberg, M., Ph.D., National Institutes of Health, Rockville, Maryland

SEMINARS

Bekiranov, S., University of Virginia, Charlottesville: Analyzing Tag4 arrays.
Hinnebusch, A., National Institutes of Health, Bethesda, Maryland: Nitogren regulation of gene expression.
Hollingsworth, N., State University of New York, Stony Brook: Phosphorylation and the regulation of meiotic recombination.
Kaeberlin, M., University of Washington, Seattle: Genetics of aging.
Levin, D., Boston University, Massachusetts: Stress-induced transcriptional regulation through a novel MAPK-driven mechanism.
Lew, D., Duke University, Durham, North Carolina: Cell polarity in yeast.

Ramanathan, S., Harvard University, Cambridge, Massachusetts: Using Bayesian statistics to uncover information about proteins and networks.
Rusche, L., Duke University, Durham, North Carolina: Evolution of transcription silencing.
Strathern, J., National Cancer Institute, Frederick, Maryland: Detection of transcription errors.
Thorner, J., University of California, Berkeley: A signaling pathway for maintenance of plasma membrane lipid homeostasis.
Troyanskaya, O., Princeton University, New Jersey: Integrating large-scale data.
Whiteway, M., National Research Council of Canada, Montreal, Quebec: Transcriptional rewiring in fungi.

Stem Cells

Stem cells construct organs and tissues in development. They sustain tissues in the adult and restore them after injury. Because of these properties, isolating and manipulating stem cells has become a major new element in biomedical science. This workshop course covered a series of biological subjects relating to stem cells and regenerative medicine. Topics included embryology and development, reprogramming, degenerative disease, cancer stem cells, and human genetics.

This 10-day-long discussion course brought together leading researchers in the stem cell field with a small group of international students. The purpose of the workshop was to provide participants with an opportunity to achieve an advanced understanding of the scientific and clinical importance of stem cells. A major aim of the workshop was to discuss, in considerable detail, the relationship between stem cells and disease. The significance of this relationship with respect to developing new approaches to treating and understanding human disease was explored.

PARTICIPANTS

Baridi, A., B.S., Cambridge Research Institute, United Kingdom

Beagle, B., B.S., University of Rochester Medical School, New York

Bolanos, D., Ph.D., Universidad de los Andes, Bogota, Colombia

Elewa, A., B.S., University of Massachusetts Medical School, Worcester

Feinberg, A., Ph.D., Carnegie Mellon University, Pittsburgh, Pennsylvania

Feng, Y., B.S., Center for Molecular and Vascular Biology, Leuven, Belgium

Jaishankar, A., B.S., National Institutes of Health, Bethesda, Maryland

Lorenzo, L., B.S., University of Maryland, Baltimore

Marrus, S., Ph.D., Washington University School of Medicine, St. Louis, Missouri

Micali, N., Ph.D., National Institute of Neurological Disorders and Stroke/NIH, Bethesda, Maryland

Schneider, J., Ph.D., Vanderbilt University Medical Center, Nashville, Tennessee

Talchai, C., B.S., Columbia University, New York

Tang, T., Ph.D., National Institute of Mental Health/NIH, Bethesda, Maryland

Tsang, S.N., Ph.D., Columbia University, New York

Turco, M., B.S., European Institute of Oncology (IFOM-IEO Campus), Milano, Italy

Vicente-Dueñas, C., Ph.D., Instituto de Biología Molecular y Celular del Cáncer, Salamanca, Spain

Yeung, M., B.S., Karolinska Institutet, Stockholm, Sweden

SEMINARS

Hadjantonakis, K., Memorial Sloan-Kettering Cancer Center, New York: Live imaging cell dynamics in the early mouse embryo.

Hochedlinger, K., Massachusetts General Hospital, Boston: Equivalency of ESCs and iPSCs?

Johnston, L., Columbia University, New York: Social interactions among growing cells: Promoting tissue fitness.

Lee, J., Massachusetts General Hospital, Boston: The X as a model to study stem cell biology and reprogramming.

Lemischka, I., Mt. Sinai School of Medicine, New York: Pursuing pluripotency from a systems biology perspective.

McKay, R., National Institutes of Health, Bethesda, Maryland: Stem cells in development and disease.

Ohlstein, B., Columbia University Medical Center, New York: Investigating the role of stem cells in tissue homeostasis and organogenesis.

Reddien, P., Whitehead Institute, Cambridge, Massachusetts: Planarians as a model for the study of stem cells and regeneration.

Rich, J., Cleveland Institute, Ohio: The evolving understanding of cancer stem cells.

Robertson, E., University of Oxford, United Kingdom: Cell lineage specification and patterning in the pre- and early postimplantation embryo.

Shen, M., Columbia University Medical Center, New York: Prostate development, stem cells, and cancer.

Studer, L., Memorial Sloan-Kettering Cancer Center, New York: Modeling neural development and disease in human pluripotent stem cells.

Tesar, P., Case Western University, Cleveland, Ohio: Derivation and utilization of distinct pluripotent stem cell states.

Tlsty, T., University of California School of Medicine, San Francisco: Mechanisms of epigenetic plasticity in stem cells and cancer.

Xiao, A., Yale School of Medicine, New Haven, Connecticut: Investigating chromatin structure in early embryonic development.

Caenorhabditis elegans

October 11–26

INSTRUCTORS   **B. Conradt,** Dartmouth Medical School, Hanover, New Hampshire
     E. Lambie, Dartmouth College, Hanover, New Hampshire
     E. Miska, University of Cambridge, United Kingdom
     M. Zhen, Samuel Lunenfeld Research Institute, Toronto, Canada

ASSISTANTS   **L. Barbier,** Samuel Lunenfeld Research Institute, Toronto, Canada
     M. Po, University of Toronto, Canada
     S. Rolland, Dartmouth Medical School, Hanover, New Hampshire
     A. Sapetschnig, University of Cambridge, United Kingdom

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis and reverse genetic approaches. A major goal was to teach students how to successfully exploit the information generated by the *C. elegans* genome project. The course was suited both for those who have a current training in molecular biology and some knowledge of genetics, but have no experience with *C. elegans*, as well as students with some prior worm experience who wished to expand their repertoire of expertise. The following topics were covered both in the laboratory and by lectures from experts in the field: worm pushing, *C. elegans* databases and worm bioinformatics, anatomy and development, forward genetics, chemical and transposon mutagenesis, generation of transgenic animals, expression pattern analysis, reverse genetics, construction and screening of deletion libraries, and RNA inactivation. The course was designed to impart sufficient training to students in the most important attributes of the *C. elegans* system to enable students to embark on their own research projects after returning to their home institutions.
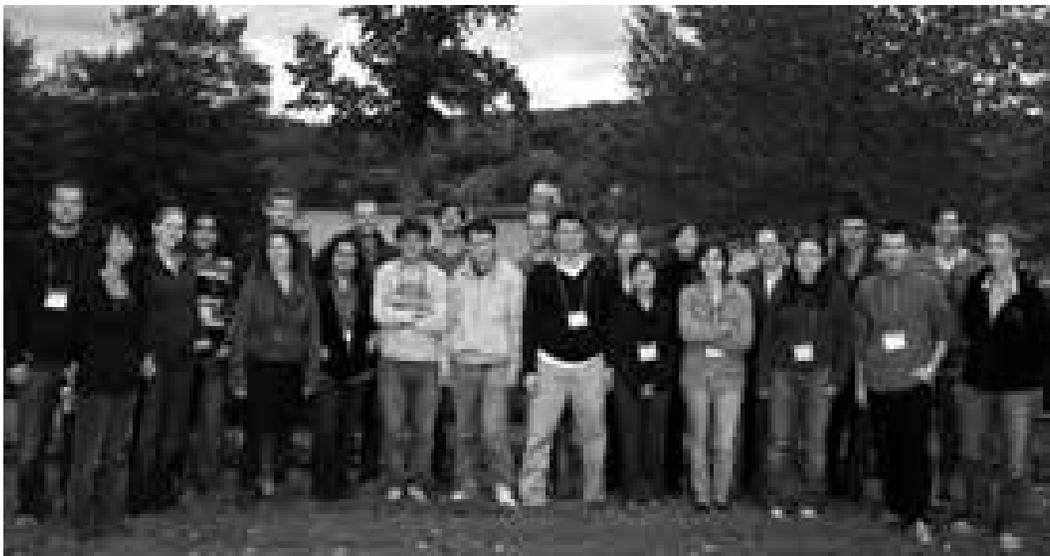
PARTICIPANTS

Baas, S., B.S., Vanderbilt University Medical Center, Nashville, Tennessee

Beets, I., M.S., K.U. Leuven, Belgium

Buzzi, L., B.Sc., Fundacion Instituto Leloir, Buenos Aires, Argentina

Cottee, P., Ph.D., The University of Alabama, Birmingham

Farhadifar, R., Ph.D., Harvard University, Cambridge, Massachusetts

Hermand, D., Ph.D., FUNDP, Namur, Belgium

Joyce, A., B.S., Washington University School of Medicine, St. Louis, Missouri

Kemeny, S., Ph.D., Columbia University, New York

Lim, M., B.A., University of Pennsylvania/CHOP, Philadelphia

Liu, Z., B.S., Chinese Academy of Science, Beijing, China

Marquis, B., M.S., National Institute of Standards & Technology, Gaithersburg, Maryland

Mi-Mi, L., B.S., State University of New York Upstate Medical University, Syracuse

Rayes, D., Ph.D., Instituto de Investigaciones Bioquimicas (NIBIBB), Bahia Blanca, Argentina

Roux, A., Ph.D., University of California, San Francisco

Tran, P., Ph.D., University of Pennyslvania, Philadelphia

van Zon, J., Ph.D., Massachusetts Institute of Technology, Cambridge

SEMINARS

Ausubel, F., Massachusetts General Hospital, Boston: The *C. elegans* immune defense response.

Besseréau, J.-L., Ecole Normale Superieure-INSERM, Paris, France: Deconstructing the *C. elegans* neuromuscular junction (with the help of transposons).

Fraser, A., The Wellcome Trust Sanger Institute, Hinxton, United Kingdom: RNAi screening in *C. elegans*.

Goodman, M., Stanford University, California: Using *C. elegans* to deconstruct touch sensation.

Hall, D., Albert Einstein College of Medicine, Bronx, New York: Introduction on *C. elegans* anatomy. Modern EM methods for nematode.

Hobert, O., Columbia University College of Physicians & Surgeons, New York: Of worm neurons and machines.

Hunter, C., Harvard University, Cambridge, Massachusetts: The discovery and genetic analysis of systemic RNAi.

Jin, Y., University of California, San Diego, La Jolla: *C. elegans* axon regeneration.

Koelle, M., Yale University School of Medicine, New Haven, Connecticut: Chloride transporters that regulate inhibitory neurotransmission in *C. elegans*.

Kornfeld, K., Washington University School of Medicine, St. Louis, Missouri: *C. elegans* as a model for aging research.

Roy, P., Mt. Sinai-Samuel Lunenfeld Research Institute, Toronto, Canada: *C. elegans* as a platform for chemical biology.

Schafer, W., University of California, San Diego, La Jolla

Schedl, T., Washington University School of Medicine, St. Louis, Missouri: The germline stem cell versus meiotic development decision in *C. elegans*.

Schnabel, R., Institut fuer Genetik, Brainschweig, Germany: A practical approach to multi-channel 4D microscopy. Phainothes and pattern formation by cell focusing in the *C. elegans* embryo.

Sommer, R., Max-Planck Institute for Developmental Biology, Tubingen, Germany: Vulva development. Vulva induction in *C. elegans* and *Pristiochus pacificus*: A mechanistic comparison.

Programming for Biology

INSTRUCTORS
S. Lewis, Lawrence Berkeley National Laboratory, Berkeley, California
S. Prochnik, DOE–Joint Genome Institute/University of California, Berkeley
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware

ASSISTANTS
J. Chia, Cold Spring Harbor Laboratory
E. Lee, Lawrence Berkeley National Laboratory, Berkeley, California
J. Leipzig, DuPont Experimental Station, Wilmington, Delaware
D. Messina, Stockholm University, Sweden
S. Robb, University of Utah, Salt Lake City
J. Thomason, Cold Spring Harbor Laboratory
K. Youens-Clark, Cold Spring Harbor Laboratory

A computer is an indispensable tool for biological research, but the mere use of web-based tools alone is not enough for today's biologist who needs to work with data from myriad sources in disparate formats. This need will become ever more important as new technologies accelerate the exponential rate of acquiring new growth biological data. Designed for students and researchers with little or no prior programming experience, this 2-week course gave biologists the bioinformatics skills necessary to exploit this abundance of biological data.

The course is based around the Perl scripting language, because of its ease of learning and its incredible wealth of ready-built code modules (e.g., bioperl) designed to solve common biological problems. Starting with introductory coding, and continuing with a survey of available biological libraries and practical topics in bioinformatics, students end by learning how to construct and run powerful and extensible analysis pipelines in a straightforward manner. The course combines formal lectures with hands-on sessions in which students work to solve problem sets covering common

scenarios in the acquisition, validation, integration, analysis, and visualization of biological data. For their final projects, which run during the second week of the course, students posed problems using their own data and worked with each other and the faculty to solve them.

PARTICIPANTS

Abu-Ali, G., Ph.D., U.S. Food & Drug Administration, Laurel, Maryland

Akhtar-Zaidi, B., B.A., Case Western Reserve University, Cleveland, Ohio

Ameres, S., Ph.D., University of Massachusetts Medical School, Worcester

Cristancho, M., B.Sc., National Center for Coffee Research, Chinchina, Columbia

David, D., Ph.D., University of California, San Francisco

Delgado Vega, A.M., M.D., Uppsala University, Sweden

Frank, M., B.A., Cornell University, Ithaca, New York

Hakhverdyan, Z., B.A., The Rockefeller University, New York

Hall, A., Ph.D., University of Liverpool, United Kingdom

Lopatina, A., B.S., Russian Academy of Sciences, Moscow, Russia

Lowe, I., M.S., University of California, Davis

Lowry, D., Ph.D., University of Texas, Austin

Ni, Z., Ph.D., Cornell University, Ithaca, New York

O'Donnell, A., Ph.D., Columbia University, New York

Onishi-Seebach, M., Ph.D., European Molecular Biology Laboratory, Heidelberg, Germany

Polymenidou, M., Ph.D., University of California, San Diego

Robertson, L., Ph.D., U.S. Geological Survey, Kearneysville, West Virginia

Rohland, N., Ph.D., Harvard Medical School, Cambridge, Massachusetts

Rynearson, S., B.S., Eccles Institute of Human Genetics, Salt Lake City, Utah

Sandberg, J., M.Sc., Royal Institute of Technology, Stockholm, Sweden

Schorn, A., Ph.D., Cold Spring Harbor Laboratory

Teng, G., Ph.D., Yale University, New Haven, Connecticut

SEMINARS

Begovic, E., University of California, Berkeley: Molecular evolution I. Molecular evolution II.

Cain, S., Ontario Institute for Cancer Research, Medina, Ohio: Gbrowse.

Deschamps, S., Pioneer Hi-Bred International,Wilmington, Delaware: Deep sequencing I.

Hide, W., Harvard University, Boston, Massachusetts: Gene expression.

Leipzig, J., DuPont, Wilmington, Delaware: HTML. Deep sequencing II. Deep sequencing and assembly. Introduction to R.

Lewis, S., Lawrence Berkeley National Laboratory, California: Gene ontologies.

McCombie, W., Cold Spring Harbor Laboratory: Genetic variation and deep sequencing. DBI.

McKay, S., University of Arizona, Tucson: Database design and SQL. DBI. CGI.

Messina, D., Stockholm Bioinformatics Centre, Sweden: Perl VI, subroutines, and modules. Perl IV–References.

Pearson, W., University of Virginia, Charlottesville: BLAST and sequence alignment. BLAST workshop.

Prochnik, S., DOE–Joint Genome Institute, Walnut Creek, California: Perl VII (object-oriented programming). Perl pipelines.

Robb, S., University of Utah, Salt Lake City: Bioperl I. Bioperl II.

Thomas, P., SRI International, Menlo Park, California: Gene expression.

Tisdall, J., DuPont Experimental Station, Wilmington, Delaware: Perl I. Perl III (arrays, hashes). Scientific computing. Perl V–Regular expressions.

X-Ray Methods in Structural Biology

October 11–26

INSTRUCTORS   **W. Furey,** V.A. Medical Center and University of Pittsburgh, Pennsylvania
     G. Gilliland, Centocor, Inc., Radnor, Pennsylvania
     A. McPherson, University of California, Irvine
     J. Pflugrath, Rigaku Americas Corporation, The Woodlands, Texas

ASSISTANT   **G. Obmolova,** Centocor, Inc., Radnor, Pennsylvania

Crystallography and X-ray diffraction yield a wealth of structural information unobtainable through other methods. This intensified laboratory/computational course focused on the major techniques used to determine the three-dimensional structures of macromolecules. It was designed for scientists with a working knowledge of protein structure and function, but who are new to macromolecular crystallography. Topics covered included basic diffraction theory, crystallization (proteins, nucleic acids, and complexes), crystal characterization, X-ray sources and optics, synchrotrons, crystal freezing, data collection, data reduction, multiple isomorphous replacement, multiwavelength anomalous diffraction, molecular replacement, solvent flattening, noncrystallographic symmetry averaging, electron density interpretation, molecular graphics, structure refinement, structure validation, coordinate deposition, and structure presentation. Participants learned through extensive hands-on experiments. One or more proteins were crystallized and the structure(s) determined by several methods, in parallel with lectures on theory and informal discussions behind the techniques. Applicants were familiar with the creation and editing of simple text files on Linux workstations using a screen-based editor (either vi or emacs).

PARTICIPANTS

Cheng, H.-C., B.S., University of California, San Francisco
Elmlund, D., M.S., Stanford University, Palo Alto, California
Engel, C., M.Sc., Ludwig-Maximilians–Universistät, Munich, Germany
Fagan, C., B.S., Emory University, Atlanta, Georgia
Festin, G., B.S., University of California, Irvine
Gallagher, C., B.Sc., University of California/HHMI, San Francisco
Hausmann, J., M.S., Netherlands Cancer Institute, Amsterdam, The Netherlands
Koh, J., Ph.D., HHMI at The Rockefeller University, New York
Lin, J., Ph.D., University of Nebraska, Lincoln

Mattle, D., M.Sc., Aarhus University, Denmark
Miller, A., B.A., Memorial Sloan-Kettering Cancer Center, New York
Murray, L., Ph.D., Yale University, New Haven, Connecticut
Rothberg, B., Ph.D., Temple University School of Medicine, Philadelphia, Pennsylvania
Stevenson, H., B.S., University of Pittsburgh, Pennsylvania
Tumbale, P., Ph.D., National Institute of Environmental Health Sciences/NIH, Research Triangle Park, North Carolina
Wang, K., Ph.D., Oregon Health & Sciences University, Portland

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Adams, P., Lawrence Berkeley Laboratory, California: Introduction to PHENIX (and CNS). Structure refinement.
Cohen, A., Stanford Synchrotron Radiation Laboratory, California: Remote synchrotron data collection.
Einspahr, H., IUCr Journals, Chester, United Kingdom: Acta crystallographica F.
Emsley, P., University of York, United Kingdom: Model-building tools in coot.
Furey, W., V.A. Medical Center and University of Pittsburgh, Pennsylvania: Anomalous data collection consideration. Patterson group therapy. Isomorphous replacement and anomalous scattering. Solvent flattening/phase combination, Noncrystallographic symmetry averaging. MAD phasing: A classical approach. Solving structures with BnP.
Furukawa, H., Cold Spring Harbor Laboratory: Structure and function of NMDA receptors.
Gilliland, G., Centorcor Inc., Radnor, Pennsylvania: Course overview: The structure determination of biological macromolecules.
Joshua-Tor, L., Cold Spring Harbor Laboratory: DNA translocation in a replicative hexameric helicase.
Kleywegt, G., EMBL-EBI, Cambridge, United Kingdom: Just because it's in Nature, doesn't mean it's true… (macromolecular structure validation).
McPherson, A., University of California, Irvine: Crystallization of macromolecules I. Crystallization of macromolecules II. Symmetry, periodicity, unit cells, space groups, Miller planes, and lattices. Waves, vectors, and complex numbers. Fundamental diffraction relationships and Bragg's law. Diffraction patterns, reciprocal space, and Ewald's sphere. Fourier transforms and the electron density equation. Patterson methods. Crystal growth mechanisms. Heavy atoms and anomalous scatterers.

Perrakis, A., Netherlands Cancer Institute, Amsterdam, The Netherlands: Automated model building and refinement with ARP/WARP.
Pflugrath, J., Rigaku Americas Corporation, The Woodlands, Texas: Data collection: Design and setup. Cryocrystallography. HKL2000 tutorial. Scaling and merging synchrotron data.
Read, R., University of Cambridge, United Kingdom: Using SAD data in Phaser. Molecular replacement: New structures from old.
Richardson, D., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using MolProbity.
Richardson, J., Duke University Medical Center, Durham, North Carolina: Structure presentation.
Schalch, T., Cold Spring Harbor Laboratory: Macromolecular representation and animation using PyMOL/eMovie.
Sweet, R., Brookhaven National Laboratory, Upton, New York: A slightly different view of fundamental crystallography. X-ray sources and optics. Scaling and merging synchrotron data.
Terwilliger, T., Los Alamos National Laboratory, New Mexico: Automated structure solution and model building.
Tronrud, D., University of Oregon, Eugene: Macromolecular refinement I. Macromolecular refinement II. Difference electron density maps. CSI CSHL: Forensic crystallography.
Westbrook, J., Rutgers/The State University of New Jersey, Piscataway: Automating PDB deposition.
Yeh, J., University of Pittsburgh, Pennsylvania: Approaches for membrane protein crystallization.

Advanced Sequencing Technologies and Applications

INSTRUCTORS   **E. Mardis,** Washington University School of Medicine, St. Louis, Missouri
G. Marth, Boston College, Chestnut Hill, Massachusetts
W. McCombie, Cold Spring Harbor Laboratory
A. Quinlan, University of Virginia, Charlottesville
M. Zody, Broad Institute, Cambridge, Massachusetts

ASSISTANTS   **A. Farrell,** Boston College, Chestnut Hill, Massachusetts
M. Kramer, Cold Spring Harbor Laboratory
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
S. McGrath, Washington University School of Medicine, St. Louis, Missouri
J. Walker, Washington University School of Medicine, St. Louis, Missouri

During the last decade, large-scale DNA sequencing has markedly impacted the practice of modern biology and is beginning to affect the practice of medicine. With the recent introduction of several next-generation sequencing technologies, costs and timelines have been reduced by orders of magnitude, facilitating investigators to conceptualize and perform sequencing-based projects that heretofore were cost- and infrastructure-prohibitive. Furthermore, the application of these technologies to answer questions previously not experimentally approachable is broadening their impact and application.

This intensive 2-week course explored applications of next-generation sequencing technologies, with a focus on commercially available platforms. Students were instructed in the detailed operation of several next-generation and third-generation sequencing platforms, including sample preparation procedures, general data handling through pipelines, and in-depth data analysis. A diverse range of applications and analyses were explored, including RNA-Seq, de novo DNA sequencing, assembly

of bacterial genomes, and human microbiome sample analysis, among others. Guest lecturers highlighted their own applications of these technologies and focused on the significant bioinformatics-based analysis component of analyzing massively parallel sequencing-derived data sets.

PARTICIPANTS

Ainsley, J., Ph.D., Tufts University, Boston, Massachusetts

Andersen, H., Ph.D., University of Southern Denmark, Odense, Denmark

Bangarusamy, D., Ph.D., King Abdullah University of Science and Technology, Thuwal, Saudi Arabia

Belton, J.-M., B.S., University of Massachusetts Medical School, Worcester

Cui, Y., Ph.D., University of Kansas Medicine Center, Kansas City

Harbst, K., M.Sc., Lund University, Sweden

Ingles, S., Ph.D., University of Southern California, Los Angeles

Joseph, V., Ph.D., Memorial Sloan-Kettering Cancer Center, New York

Karmin, M., M.Sc., University of Tartu and Estonian Biocentre, Tartu, Estonia

Lagier-Tourenne, C., M.D., University of California, San Diego, La Jolla

Liu, P., Ph.D., National Heart, Lung, and Blood Institute/NIH, Bethesda, Maryland

Maestro, R., Ph.D., CRO Aviano National Cancer Institute, Aviano (PN), Italy

Mafofo, J., Ph.D., University of the Western Cape, Cape Town, South Africa

Marie, C., Ph.D., University of Virginia, Charlottesville

Salomon, M., B.S., University of Florida, Gainesville

Zhidkov, I., M.Sc., Ben Gurion University of the Negev, Beer Sheva, Israel

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Church, D., DHHS/NIH/NLM/NCBI, Bethesda, Maryland: Status of the human and mouse reference genomes and the short-read archive.

Eichler, E., University of Washington, Seattle: Structural variation in human genomes and its association to genetic disease.

Eid, J., Pacific Biosciences Incorporated, Menlo Park, California: The Pacific biosciences platform.

Leamon, J., Ion Torrent, Guilford, Connecticut: The ion torrent sequencing technology.

Mardis, E., Washington University School of Medicine, St. Louis, Missouri: Next-generation cancer genomics.

McPherson, J., Ontario Institute for Cancer Research, Toronto, Canada: Cancer genomics at the Ontario Institute for Cancer Research.

Navin, N., Cold Spring Harbor Laboratory: Genomic heterogeneity in breast cancer.

Schatz, M., Cold Spring Harbor Laboratory: Cloud computing with next-generation sequencing data.

Weinstock, G., The Genome Center at Washington University School of Medicine, St. Louis, Missouri: Metagenomics and the human microbiome project.

Immunocytochemistry, In Situ Hybridization, Super-Resolution, and Live Cell Imaging

October 13–26

INSTRUCTORS **V. Allan,** University of Manchester, United Kingdom
      K. Hu, Indiana University, Bloomington
      J. Murray, University School of Medicine, Philadelphia, Pennsylvania

CO-INSTRUCTOR **J. Peng,** Stanford University, California

ASSISTANTS **Y.-C. Hwang,** University of California, Santa Cruz
      A. Oguro-ando, University of California, Los Angeles

This course focused on specialized techniques in microscopy, in situ hybridization, immunocyto-chemistry, and live cell imaging related to localizing DNA, RNA, and proteins in fixed cells as well as protein and RNA dynamics in living cells. The course emphasized the use of the latest equipment and techniques in fluorescence microscopy, including confocal laser-scanning microscopy; decon-volution methods; several super-resolution methods including structured illumination, STORM, and PALM; digital image processing; and time-lapse imaging of living specimens. The course was

designed to present students with state-of-the-art technology and scientific expertise in the use of light microscopy to address basic questions in cellular and molecular biology. The course was designed for the molecular biologist who is in need of microscopic approaches and for the cell biologist who is not familiar with the practical application of the advanced techniques presented in the course. Among the methods presented were the preparation of tagged nucleic acid probes; fixation methods; detection of multiple DNA sequences in single nuclei or chromosome spreads; cellular localization of RNA; localization of nucleic acids and proteins in the same cells; use of a variety of reporter molecules and nonantibody fluorescent tags; indirect antibody labeling; detection of multiple proteins in a single cell; labeling antibodies with two fluorophores for STORM; the use of GFP variants to study protein expression in living cells by conventional microscopy and PALM; and use of photo-activable and -switchable fluorescent proteins for studying localization and dynamics. In each method, several experimental protocols were presented, allowing the students to access the relative merits of each and relate them to their own research. Students were encouraged to bring their own nucleic acid, protein, or antibody probes to the course, which were used in addition to those provided by the instructors. The laboratory exercises were supplemented with lectures given by invited distinguished scientists, who presented up to the minute reports on current methods and research using the techniques being presented.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Albania, L., M.S., University of Padua, Padova, Italy
Aspalter, I., M.S., London Research Institute, United Kingdom
Badding, M., B.S., University of Rochester Medical Center, New York
Baluch, D., Ph.D., Arizona State University, Tempe
Chi, R., Ph.D., University of Miami, Florida
Cicotte, K., B.S., University of New Mexico, Albuquerque
Elsing, A., M.S., Abo Akademi University, Turku, Finland
Francis, D., B.A., Mount Sinai School of Medicine, New York

Fu, X., Ph.D., Children's National Medical Center, Washington, D.C.
Kanaoka, M., Ph.D., Nagoya University, Japan
Paszek, P., Ph.D., University of Liverpool, United Kingdom
Pedersen, S., Ph.D., University of Copenhagen, Denmark
Rao, A., Ph.D., Carnegie Mellon University, Pittsburgh, Pennsylvania
Singh, K., Ph.D., Cleveland Clinic, Ohio
Zhao, Y., Ph.D., UMDNJ–New Jersey Medical School, Newark

SEMINARS

Allan, V., University of Manchester, United Kingdom: Immunocytochemistry.
Davidson, M., The Florida State University, Tallahassee: A library of fluorescent proteins for live cell imaging.
Day, R., Indiana University School of Medicine, Indianapolis: Visualizing protein interactions using FLIM and FRET.
Dernberg, A., University of California/HHMI Berkeley: Chromosomes dynamics during meiosis.
Goodwin, P., Applied Precision, Inc., Issaquah, Washington: Introduction to structured illumination methods.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Introduction to light and fluorescence microscopy. Digital detectors and digital imaging fundamentals. Microscopy of thick specimens.

North, A., The Rockfeller University, New York: Immuno-cytochemistry on tissue sections: principles and practice.
Ried, T., National Cancer Institute/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Shroff, H., and York, A., National Institutes of Health, Bethesda, Maryland: Super-resolution methods for cell biology.
Spector, D., Cold Spring Harbor Laboratory: Visualizing gene expression/silencing in living cells.

Computational and Comparative Genomics

November 3–9

INSTRUCTORS   **W. Pearson,** University of Virginia, Charlottesville
                             L. Stubbs, University of Illinois, Urbana

ASSISTANTS   **K. Nowick,** Max-Planck Institute for Moelcular Genetics, Berlin, Germany
                             D. Triant, Louisana State University, Baton Rouge

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 short-read "next-gen" sequencing data, identification of conserved signals in aligned and unaligned sequences, regulatory element and motif recognition, and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data. The course made extensive use of local WWW pages to present problem sets and the computing tools to solve them. Advanced programming skills were not required. The course was designed for biologists seeking advanced training in biological sequence and genome analysis, computational biology core resource directors and staff, and scientists in other disciplines, such as computer science, who wish to survey current research problems in biological sequence analysis.
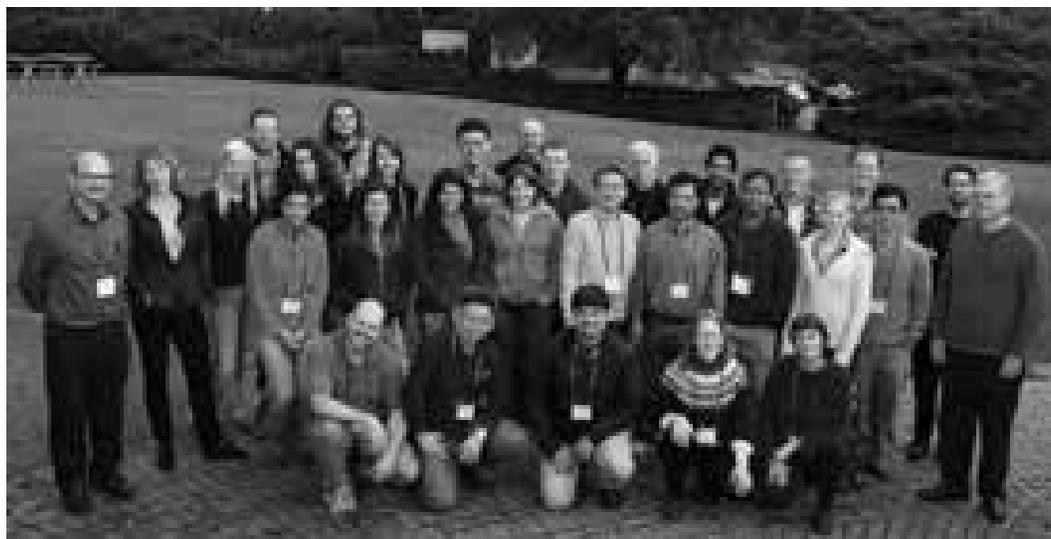
PARTICIPANTS

Blech-Hermoni, Y., B.S., Cleveland Clinic Foundation, Ohio
Burge, C., Ph.D., Cornell University, Ithaca, New York
Cabrera, A., B.S., The Ohio State University, Wooster
Correa, A., Ph.D., Florida International University, N. Miami
Dey, N., B.A., University of California, San Francisco
Egan, J., Ph.D., Mayo Clinic, Scottsdale, Arizona
Farrar, M., B.S., Howard Hughes Medical Institute, Ashburn, Virginia
Karki, A., B.S., South Dakota State University, Brookings
Kavanaugh, L., Ph.D., Syngenta Biotechnology, Inc., Research Triangle Park, North Carolina
Kim, J., Ph.D., Asan Medical Center, Seoul, South Korea
Lim, H.-C., B.Env.S., University of Illinois, Urbana-Champaign
Madissoon, E., M.Sc., Karolinska Institute, Stockholm, Sweden

Rea, T., M.S., University of Michigan, Ann Arbor
Singh, S., Ph.D., University of Minnesota, St. Paul
Stranneheim, H., M.S., SCiLifeLab-KTH, Stockholm, Sweden
Summers, H., B.S., Cornell University, Ithaca, New York
Tincopa Marca, L.-R., M.S., International Potato Center, Lima, Peru
Tripathi, P., M.S., South Dakota State University, Brookings
Tsoi, S., Ph.D., University of Alberta, Edmonton, Canada
Tutaj, M., M.S., Medical College of Wisconsin, Milwaukee
Unckless, R., M.S., University of Rochester, New York
Wijeratne, A., Ph.D., Ohio State University, Wooster
Yap, E., D.Phil., DSO National Labs, Singapore

SEMINARS

Edwards, R., San Diego State University, California: Metagenomics goals, strategies, and tools. Introduction to metagenomics projects. Metagenomics II–Analysis pipelines.
Mackey, A., University of Virginia, Charlottesville: Analysis of next-generation sequence data I. Introduction to genome biology projects.
Overduin, B., EMBL–European Bioinformatics Institute, Hinxton, Cambridge, United Kingdom: The ENSEMBL database of genomes I. ENSEMBL/BioMart.
Pearson, W., University of Virginia, Charlottesville: Introduction and overview. Protein evolution and sequence

similarity searching. Similarity searching II–Strategies. Suboptimal global alignments. Sensitive sequence comparison, PSSMs, and HMMs.
Sinha, S., University of Illinois, Urbana-Champaign: Identifying functional elements I. Functional elements II.
Stubbs, L., University of Illinois, Urbana: Introduction to genome biology. Genome biology II.
Taylor, J., Emory University, Atlanta, Georgia: Galaxy for high-throughput analysis.
Wold, B., California Institute of Technology, Pasadena: Next-generation biology.

Phage Display of Proteins and Peptides

November 9–22

INSTRUCTORS   **C. Barbas,** The Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, San Diego, La Jolla

ASSISTANTS   **F. Axelsson,** Lund University, Sweden
S. Kacir, University of Pennsylvania, Philadelphia
K. Noren, New England BioLabs, Ipswich, Massachusetts

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 libraries. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of Fab fragments expressed in *Escherichia coli* were also covered. Epitopes of a mouse monoclonal antibody were selected from a peptide display library and characterized.

The lecture series, presented by a number of invited speakers, emphasized PCR of immunoglobulin genes, the biology of filamentous phage, the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, directed protein evolution, retroviral and cell display libraries, the immunobiology of the antibody response, and recent results on the use of antibodies in therapy. The theory and practical implications for selection from phage displayed libraries of random peptides, cDNA products, and semisynthetic proteins was also explored.

PARTICIPANTS

Acharya, J., Ph.D., National Cancer Institute, Frederick, Maryland
Batra, G., Ph.D., University of Turku, Finland
Ching, A., M.Sc., Butantan Institute, Sao Paulo, Brazil
Chiu, E., M.S., Mount Sinai Hospital, Toronto, Canada
Darris, C., B.S., Tennessee State University, Nashville
Derda, R., Ph.D., Harvard University, Cambridge, Massachusetts
Dubiley, S., Ph.D., IBG RAS, Moscow, Russia
Hasan, A., M.D., Memorial Sloan-Kettering Cancer Center, New York

Lew, S., Ph.D., Columbia University, New York
Lima, S., M.Sc., University of São Paulo, Brazil
Lindberg, H., M.Sc., KTH, Stockholm, Sweden
Mohr, A., M.Sc., University of Copenhagen, Denmark
Novinger, L., B.S., University of Vermont College of Medicine, Burlington
Prudencio, C., Ph.D., University of São Paulo, Brazil
Qian, Y., Ph.D., University of North Carolina, Chapel Hill
Sanchez, A., M.S., Biokit, S.A., Barcelona, Spain

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Barbas, C.S., Scripps Research Institute, La Jolla, California: Functional antibodies and instant immunity.
Dreier, B., University of Zurich, Switzerland: Ribosome display libraries.
Ladner, R., Dyax Corporation, Ijamsville, Maryland: Phage display of peptides and their development at therapeutics.
Noren, C., New Englands Biolabs, Ipswich, Massachusetts: Functional peptide libraries.
Rader, C., National Institutes of Health, Bethesda, Maryland: Toward better monoclonal antibodies for cancer therapy: From antigen discovery to antibody engineering.

Siegel, D., University of Pennsylvania, Philadelphia: Cell-surface selection of phage-displayed antibodies.
Sidhu, S., University of Toronto, Ontario, Canada: Phage display of synthetic antibodies and SH2 domains.
Silverman, G., University of California, San Diego: Overview of the adaptive immune system.
Stahl, S., Royal Institute of Technology, Stockholm, Sweden: Phage display of affibodies.

The Genome Access Course

TRAINERS U. **Hilgert,** Cold Spring Harbor Laboratory
G. **Howell,** The Jackson Laboratory
B. **King,** Mount Desert Island Biological Laboratories
L. **Reinholdt,** The Jackson Laboratory

This course was an intensive 2-day introduction to bioinformatics that was held twice in 2010 and trained almost 40 participants in total. The core of the course was designed to cover the manipulation and analysis of sequence information. The course was broken into modules designed to give a broad overview of a given topic, with ample time for examples chosen by the instructors. Each module included three parts, consisting of a discussion of theory and methods, coverage of software and web resources, and use of selected tools with examples (including those supplied by the students). The modular design allowed the instructors to tailor the presentation to the interests of the students. Modules included Electronic Sequence Information; Pairwise Sequence Comparisons; Multiple Sequence Alignments; Gene Prediction; Genome Analysis; Sequence Variation; Protein Classification and Structural Analysis; Proteomics; and Phylogenetic Analysis. Applications to the course were open to all on a first-come-first-served basis, subject to basic eligibility requirements. The course was held at the Laboratory's Genome Research Center at Woodbury located seven miles south of the main Laboratory campus. Each student was provided with a PC laptop with wireless modem for the duration of the course. Students were encouraged to supply problem sets and sequences of interest to the trainers for possible incorporation as examples in the modules. Materials were made available on the web and students continued to ask questions of the trainers as they applied what they had learned in their individual endeavors.

April 27–29

Chowanadisai, W., Marine Biological Laboratory, Woods Hole, Massachusetts

Dolan, P., University of Rochester/University of Alabama, Birmingham, New York

Ernst, P., Dartmouth Medical School, Hanover, New Hampshire

Eswarakumar, V., Yale University, New Haven, Connecticut

Faucher, S., Public Health Agency of Canada, Ottawa

Gebhardt, C., Harvard University, Cambridge, Massachusetts

Ghiban, E., Cold Spring Harbor Laboratory

Gierszewska, M., Cold Spring Harbor Laboratory

Glaser, R., Stevenson University, Maryland

Kucera, S., University of Tampa, Florida

Li, Z., The Rockefeller University, New York

Lu, S., Harvard University, Cambridge, Massachusetts

McFadden, S., Independent Scientific Research Advocates, Bethesda, Maryland

Sattely, E., Harvard Medical School, Boston, Massachusetts

November 16–17

Ander, B., University of California, Davis M.I.N.D. Institute, Sacramento

Boland, L., University of Richmond, Virginia

Bradley, B., Yale University, New Haven, Connecticut

Cruz, V., USDA, ARS, NCGRP, Fort Collins, Colorado

Dadras, S., University of Connecticut, Farmington

Dirk, M., Stony Brook University, New York

Dolezal, D., SUNY Upstate Medical University, Syracuse, New York

Duman, E., Stony Brook University, New York

Fowler, T., Tufts University, Boston, Massachusetts

Franco, S,. Johns Hopkins University, Baltimore, Maryland

Jurkiewicz, S., SUNY Stony Brook, New York

Kung, V., Northwestern University, Chicago, Illinois

Logue, M., Boston University School of Medicine, Massachusetts

Marko, M., Cleveland Clinic, Ohio

Ozer, E., Northwestern University, Chicago, Illinois

Panella, L., USDA, ARS, CRL, Fort Collins, Colorado

Patil, V., UMDNJ–New Jersey Medical School, Newark

Reilley, A., National Center for Genetic Resources Preservation, Fort Collins, Colorado

Richards, C., National Center for Genetic Resources Preservation, Fort Collins, Colorado

Romero, S., Yale University, New Haven, Connecticut

Stone, A., Arizona State University, Tempe

Szpara, M., Princeton University, New Jersey

Chaozhe, Y., University of Alabama, Birmingham

Zuo, L., Yale University, West Haven, Connecticut

The Laboratory acknowledges the generosity of the following companies that loaned equipment and reagents to the various courses:

SEMINARS

INVITED SPEAKER PROGRAM

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their findings. These weekly seminars keep the CSHL staff current on the latest scientific developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

	Title	Host
January		
Dr. Eric Holland, Memorial Sloan-Kettering Cancer Center	Brain tumor stem-like cells and where they live.	Grisha Enikolopov
Dr. Brian Chait, The Rockefeller University	Proteomic and nanoengineering probes of nucleocytoplasmic transport.	Leemor Joshua-Tor
Dr. Steve Henikoff, Fred Hutchinson Cancer Research Center	Histone variant dynamics and epigenetics.	Chris Vakoc
February		
Dr. David Bartel, Whitehead Institute	microRNAs and other small regulatory RNAs.	Leemor Joshua-Tor
Dr. Philip Benfey, Duke University	Development rooted in interwoven networks.	Marja Timmermans
Dr. Amy Wagers, Harvard University	Reversing dysfunction of aged stem cells.	Terri Grodzicker
March		
Dr. Hugo Bellen, Baylor College of Medicine	A forward genetic approach to study neurodegeneration.	Linda Van Aelst
Dr. Josh Sanes, Harvard University	Synaptic specificity in the visual system.	Josh Huang
Dr. Karl Deisseroth, Stanford University	Optogenetics: New developments, new applications.	Tony Zador
Dr. Lisa Coussens, University of California, San Francisco	Inflammation and cancer: Polarized immune responses regulate cancer development.	Mikala Egeblad
April		
Dr. Eric Nestler, Mount Sinai School of Medicine	Transcriptional and epigenetic mechanisms of addiction.	Grigori Enikolopov
Dr. Gary Ruvkun, Harvard University	Comprehensive identification of *C. elegans* small RNA pathway genes.	Leemor Joshua-Tor
Dr. David Tuveson, Cancer Research UK	Modeling cancer biology and medicine.	Lloyd Trotman
Dr. David Eisenberg, University of California, Los Angeles	Structural studies of the amyloid state.	Leemor Joshua-Tor
May		
Dr. Karel Svoboda, Howard Hughes Medical Institute	The neural circuits underlying somatosensation.	Tony Zador
October		
Dr. Irving Weissman, Stanford University	Normal and neoplastic stem cells.	Grigori Enikolopov
Dr. Manuel Serrano, Spanish National Cancer Research Centre	Tumor suppressors at the interface of cancer and aging.	Scott Lowe
November		
Dr. Angelika Amon, Massachusetts Institute of Technology	The consequences of aneuploidy.	Terri Grodzicker
Dr. David Lewis, University of Pittsburgh	Cortical E/I balance and cognitive dysfunction in schizophrenia.	Josh Huang
Dr. Mark Ptashne, Memorial Sloan-Kettering Cancer Center	Chromatin architecture: What determines it and what is its function?	Alex Gann

December

Dr. James Surmeier, Northwestern University Medical School	Calcium, mitochondrial oxidant stress and Parkinson's disease.	Pavel Osten
Dr. Rachel Green, Johns Hopkins University	Quality control during translation in bacteria and yeast.	Leemor Joshua-Tor

IN-HOUSE SEMINAR PROGRAM

Cold Spring Harbor Laboratory's In-House Seminars were initiated to provide a semiformal avenue for communication between the various research groups at the Laboratory. The seminars also afford a necessary opportunity for the graduate students and postgraduate staff to develop their skills in organizing, presenting, and defending their research.

Title

January

Mike Wigler — A unified genetic model for autism.

Prem Premsrirut (Lowe Lab) — SLowe mice no more: A rapid and efficient method to produce conditional transgenic mice using RNAi.

Mikel Zaratiegui (Martienssen Lab) — Centromere-binding protein B preserves genome integrity at replication forks paused by retrotransposons.

Anthony Mazurek (Stillman Lab) — Discovery of an essential role for the DEAD-box RNA helicase, DDX5, in cell cycle control and breast cancer development.

February

Florin Albeanu — Understanding olfactory neuronal circuits in the mammalian brain.

Alexander Dobin (Gingeras Lab) — STARgazing reveals novel UFTs.

Shane McCarthy (Sebat Lab) — Rare structural variants: Insights into the genetics of schizophrenia.

March

Pavel Osten — Search for neural circuit endophenotypes in mouse models of autism.

Hysell Oviedo (Zador Lab) — Form follows function: Linking circuits, connectivity, and function in the auditory cortex.

Raffaella Sordella — Molecular targeted cancer therapies: Quo vadis?

April

Glenn Turner — Population coding in the *Drosophila* mushroom body.

Michael Zhang — Machine learning and learning machines.

Alea Mills — Engineering the genome: Cancer, chromatin, and chronic behavior.

October

Chris Vakoc — Epigenetics of malignant hematopoiesis.

Fritz Henn — Toward a new pathophysiology of depression.

Mikala Egeblad — Seeing tumors in context.

November 5

James Watson — Judah Folkman, anti-angiogenesis and the curing of cancer.

Antoine Molaro and Emily Hodges (Hannon Lab) — DNA methylation dynamics across mammalian evolution and development.

Zhenxun Wang (Krainer Lab) — Investigating the mechanism of pyruvate kinase-M mutually exclusive splicing.

December

Xianfeng (Morgan) Xu (Jackson Lab) — Cell-to-cell trafficking of a plant stem cell regulator is mediated by chaperonins.

Josh Huang — Toward a genetic dissection of neural circuits in cerebral cortex: Chandeliers light up the path from epigenomes to cognition.

Shuang Ni (Stillman Lab) — ORC subunits Orc2 and Orc3 are required for stable spindle attachment at human chromosome kinetochores during mitosis.

BANBURY CENTER

BANBURY CENTER
EXECUTIVE DIRECTOR'S REPORT

The Banbury Center is now in its 33rd year and continues to be used intensively. In 2010, there were 22 science-related meetings attended by 577 participants. In addition, the Center was made available to community groups on five occasions. The Meetings and Courses Program held six lecture courses during the summer months and the Watson School of Biological Sciences used the Center for its two *Topics in Biology* courses.

Of the 577 participants, 486 (71%) were from the United States. They were drawn from 33 states, the geographical distribution reflecting the degree of biomedical research in the United States. The 91 foreign participants came from 18 countries, the majority from the United Kingdom. The ratio of male to female participants remains at 2:1.

The meeting topics were unusually wide-ranging, even for the Banbury Center. The Center has always held meetings on policy issues, issues where biomedical research is relevant to matters of so-cietal interest. The first of two such meetings in 2010 concerned science and economic policy. The American Recovery and Rein-vestment Act (ARRA) provided an extra $10.4 billion funding over a 2-year period for the National Institutes of Health (NIH) to pro-mote biomedical research through funding new projects, construc-tion, and the purchase of equipment. However, although this has been a great success, the question arises as to what will happen when the ARRA funding comes to an end. *How Can We Maintain the Stability of Biomedical Research and Development at the End of the ARRA?* examined first how NIH spent the ARRA funding and second how federal agencies, universities, research institutes, and individual investigators can manage the ending of ARRA support. Participants included officials from NIH, universities, research in-stitutes, economists, and scientists.

M. Battin, A. Asche, E. MacDonald

The second meeting, funded by the Ellison Medical Foundation, discussed *Easeful Death: 21st Century Perspectives on Assisted Suicide.* End-of-life issues involve profound legal, moral, religious, and biomedical questions and evoke intense passions. The Banbury Center is admirably suited to tackle difficult topics, although these are more usually controversies in the interpretation of scientific data. Discussions covered three broad areas. First, there was an extended discussion on whether there are distinctions to be drawn between a physician acquiescing to a patient's refusal of food and water, as-sisting in suicide by making it possible through providing materials, and the physician actively taking part in the suicide. Second, participants from Belgium and The Netherlands presented data on whether the legalization of euthanasia and assisted suicide changes the behaviors of patients and physicians, and whether the slippery slope argument carries any weight. Third, we discussed the changes in the political and legal apparatus that will have to come about before there can be acceptance of easeful death as a proper end to life. Participants included scientists, physicians, lawyers, philosophers, and religious leaders of differing opinions and although no consensus was likely to have been reached, the discussions were of remarkable quality and interest.

Turning to more typical Banbury Center topics, there were several meetings on cancer. *Energy Metabolism, the Cell Cycle, and Cancer* explored an old hypothesis on the fundamental nature of cancer in the light of modern molecular analysis. The Warburg Hypothesis was advanced by Otto Warburg in 1924 when he found that tumor cells mainly generate energy anaerobically by glycolysis

rather than by oxidative metabolism. Warburg believed that this was a fundamental characteristic of cancer cells, a thesis that fell out of fashion with the discovery of oncogenes and a focus on the genetic changes in cancer. However, in recent years links have been found between glycolysis and oncogenes and participants in this meeting reviewed these findings. For example, one session was devoted to cancer metabolism and the tumor suppressor protein p53.

A second meeting on cancer, *Tumor Microenvironment and Metastasis*, reviewed the evidence that the cancer cell is not a "renegade" cell, growing and multiplying without regard for its surroundings. On the contrary, there is increasing evidence that the behavior of a cancer cell, particularly metastasis, is influenced by its microenvironment, which includes other cells and the tumor-associated extracellular matrix. Participants examined such questions as, Is all the information needed for a cell to metastasize autonomous or does the tissue microenvironment have a role in determining this process? Does disrupting the tumor microenvironment have a positive or negative impact on metastatic potential of cancer cells? Can the microenvironment explain why many primary tumors favor secondary tumor formation in specific organs? The meeting explored what constitutes the tumor microenvironment and examined its functional impact on metastasis with specific focus on new targets for treatment of metastatic cancer.

Banbury Center has a long-standing interest in promoting research on psychiatric disorders, beginning in the early 1990s when RFLP (restriction-fragment–length polymorphism) linkage mapping was being used to try to locate genes involved in schizophrenia and depression. These and later genetic mapping strategies such as genome-wide association studies have proved disappointing and thus, it was fascinating to hold a meeting—*The Lateral Habenula: Its Role in Behavior and Psychiatric Disorders*—based on a well-defined, if poorly understood, anatomical feature of the brain. The starting point for interest in the lateral habenula was the observation that deep-brain stimulation of the area in a woman profoundly disabled by depression led to a remission that persisted as long as the stimulation continued. This meeting began with the fundamental anatomy and functional connections of the habenula before progressing to its role in cognition and how it might have a role in clinical depression.

A closely related meeting was *Linguistic Phenotypes: Toward a Biological Understanding of Language*

N. Chomsky

that explored the thesis that language can provide a window into mental function which can be exploited for the study and understanding of neuropsychiatric disorders. If linguistic performance reflects thought processes, quantitative measures defined on the basis of linguistic performance might be useful in characterizing phenotypic variability among individuals. Specific examples include autism, specific language impairment, and Williams syndrome. Schizophrenia, which includes thought disorder in its symptom list, may also be amenable to useful linguistic phenotyping. Participants included linguists and psycholinguists interested in human neurobiology and disease, as well as biologists working on autism, schizophrenia, and other neuropsychiatric disorders. We were particularly pleased that Noam Chomsky took part in the meeting.

There were two Banbury Center meetings relating to history. Mutations are essential for genetic analysis and T.H. Morgan's white-eyed *Drosophila* mutation initiated the modern era of genetics. Mutations cause inherited disorders and generate the variability on which natural selection acts. The first of our two historical meetings, *Mutagenesis: What It Means and How It Has Changed,* examined how genes and mutations were regarded in the early days of genetics and how those ideas changed with the advent of molecular genetics in the 1950s and 1960s. There were also presentations on the consequences of environmental sources of mutation: radiation and chemical carcinogens. We also discussed contemporary research on mutations, such as copy-number variation, and mutation-like phenomena in prions.

N. Federoff, C. Weisman, E. Friedberg, S. Brenner, A. Creager

Lewis Lehrman (The Lehrman Institute) wishes to encourage the use of genetic and genomic analyses to inform our knowledge of history and has established a program to foster interactions between scientists and historians. Examples of the power of such interactions are evident in studies tracking the movements of early human beings out of Africa and subsequent spread of populations throughout the globe; where and how key events in domestication of plants and animals occurred; and what DNA sequencing is telling us about our relationship with Neanderthals. Participants in *DNA, Genetics, and the History of Mankind* reviewed these and other topics. The meeting closed with a free-ranging discussion on how to promote interactions between historians and scientists and on what topics should be covered in the follow-up meeting.

The Banbury Center could not operate at its high level without the hard work of many people. The Center is especially fortunate in having Janice Tozzo and Susanne Igneri ensuring that the meetings run smoothly, and Basia Polakowski making sure that participants are welcome in Robertson House. Sonny Leute, Alvin Watson, and Fredy Vasquez look after the grounds, dealing with vast amounts of leaves in the fall and, this year, vast amounts of snow in the winter. Jon Parsons is indefatigable in handling AV requirements and Connie Brukin took the photographs which enliven the report. Culinary Services feeds our participants and Housekeeping copes admirably with the rapid turnover of guests.

Jan Witkowski
Executive Director

BANBURY CENTER MEETINGS

p53 Retreat

January 28–30

FUNDED BY   **Columbia University**, New York, New York

ARRANGED BY   **C. Prives,** Columbia University, New York, New York
                   S. Lowe, Cold Spring Harbor Laboratory, New York

The p53 protein first identified in 1979 and believed to be an oncoprotein was shown in 1989 to be a tumor suppressor. It has a central role in cell cycle control, regulation of DNA repair, and the initiation of apoptosis and is the gene most frequently mutated in cancers. p53 was named the 1993 "Molecule of the Year" by *Science.* It is not surprising then that p53 has been the subject of intensive investigation, not least by research groups at Cold Spring Harbor Laboratory (Lowe), Princeton (Levine), and Columbia (Prives and Cordon-Cardo). Members of these laboratories came to Banbury Center to report on their work and to promote interactions between the groups.

Levine Lab Presentation #1
   D. Carpizo: p53 allele-specific synthetic lethality.

Cordon-Cardo Lab Presentation #1
   O. Karni-Schmidt: New findings on p53 and its possible roles in bladder cancer and development.

Prives Lab Presentation #1
   S. Singer: Nup98, a potential tumor suppressor regulates select p53 target gene expression by a novel mechanism.

Lowe Lab Presentation #1
   C. Scuoppo: The polyamine–hypusine axis defines a tumor suppressor network in human lymphoma.

PI PLANNING MEETING

Cordon-Cardo Lab Presentation #2
   A. Jia: microRNAs in bladder cancer progression.

Levine Lab Presentation #2
   H. Mizuno: Stem cell signatures in breast cancer with p53 mutations.

Prives Lab Presentation #2
   W. Freed-Pastor: Mutant p53 prevents formation of mammary acini in three-dimensional culture.

Lowe Lab Presentation #2
   L. Dow: How to make a hairy mouse: New transgenic shRNA technologies to allow reversible loss of function phenotypes in adult mice.

Guest Lecturers
   R. Parsons: PTEN regulation in cancer: What is the contribution of p53 mutation?
   J. Manley: mRNA processing and cancer: Roles for p53 and other tumor suppressors and oncogenes.

Prives Lab Presentation #3
   C. Priest: A novel Mdm2 mutant that degrades itself preferentially over p53.

Lowe Lab Presentation #3
   Z. Zhao: p53 affects self-renewal in AML.

Wrap-up Discussion

The Lateral Habenula: Its Role in Behavior and Psychiatric Disorders

February 28–March 3

Depression is the disorder that is predicted to cause the greatest morbidity in the world by 2020, and a 2009 survey found 16% of Americans suffer from depression. Recently, deep-brain stimulation has been suggested as a possible therapy for refractory cases, and data suggest that the target of this stimulation is the lateral habenula. This is a relatively little-studied part of the brain, and given these findings, this was the right time to critically review what is known of the function of this structure, its connections, and how they function. Participants included anatomists, physiologists, neuropharmacologists, and clinicians.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Background: **J. Watson,** Cold Spring Harbor Laboratory, New York
Introduction: **F. Henn,** Brookhaven National Laboratory, Upton, New York
                         B. Li, Cold Spring Harbor Laboratory, New York

SESSION 1: The Lateral Habenula: Anatomical/Functional Connections and Cellular Biology
Chairperson: O. Hikosaka, National Eye Institute, Bethesda, Maryland

M. Herkenham, National Institute of Mental Health, Bethesda, Maryland: The anatomical connections of the habenula nuclei, with a historical perspective.

S. Sesack, University of Pittsburgh, Pennsylvania: Projections from the lateral habenula to midbrain dopamine neurons: indirect inhibitory control via the rostromedial mesopontine tegmentum.

T. Jhou, National Institute on Drug Abuse, Baltimore, Maryland: A convergence of aversion: Fear, disappointment, and inhibition in the rostromedial tegmentum (RMTg), a habenula target.

S. Haber, University of Rochester Medical Center, New York: The place of habenula in the reward circuit.

P. Shepard, University of Maryland, Baltimore: Stimulation of the lateral habenula inhibits the activity of midbrain dopamine neurons at the population level.

SESSION 2: The Lateral Habenula: Anatomical/Functional Connections, and Cellular Biology (Cont'd)
Chairperson: S. Haber, University of Rochester Medical Center, New York

R. Veh, Charité—Universitätsmedizin Berlin, Germany: Intrinsic properties and connections of the lateral habenular complex in the rat.

U. Kim, Pennsylvania State University College of Medicine, Hershey: Morphological and electrophysiological properties of the habenula.

R. Blakely, Vanderbilt School of Medicine, Nashville, Tennessee: Ironing out a relationship between serotonin and the habenula: Insights from a genetic reference mouse population

H. Okamoto, RIKEN Brain Science Institute, Wako City, Japan: Functional analysis of the dorsal habenula in zebra fish, an equivalent structure to the mammalian medial habenula.

E. Turner, Seattle Children's Research Institute, Washington: A gene regulatory program for habenula development.

SESSION 3: The Lateral Habenula and Cognitive Function
Chairperson: B. Moghaddam, University of Pittsburgh, Pennsylvania

O. Hikosaka, National Eye Institute, Bethesda, Maryland: Role of the lateral habenula in value-based decision-making.

L. Lecourtier, Louis Pasteur University, Strasbourg, France: Role of the habenula in cognitive processes, linked to its regulatory role over many neurotransmitter systems.

H. Piggins, University of Manchester, United Kingdom: Role of the habenula in the temporal regulation of brain states and behavior.

M. Ullsperger, Radboud University Nijmegen, The Netherlands: Role of the habenula in performance monitoring and cognitive control. Approaches in human and animal research.

G. Yadid, Bar-Ilan University, Ramat-Gan, Israel: Electrical stimulation of the lateral habenula for modulation of the reward system: Application to depression and addiction.

SESSION 4: The Lateral Habenula and the Neural Mechanisms Underlying Clinical Depression
Chairperson: F.A. Henn, Brookhaven National Laboratory, Upton, New York

W. Drevets, University of Oklahoma-Tulsa University School of Community Medicine: Abnormalities of habenular structure and function in mood and anxiety disorders.

J. Roiser, University College London, United Kingdom: Role of the habenula in psychiatric disorders.

A. Sartorius, Central Institute of Mental Health, Mannheim, Germany: Lateral habenula and treatment-resistant depression: Results of functional inhibition of the lateral habenula in congenitally helpless rats and in a first patient.

G. Northoff, University of Ottawa, Ontario, Canada: Subcortical regions and their relevance in emotion processing in depression.

SESSION 5: The Lateral Habenula and Animal Models of Psychiatric Disorders
Chairperson: R. Dolan, University College London, United Kingdom

F. Henn, Brookhaven National Laboratory, Upton, New York: An animal model of depression: A key to understanding the road to the habenula.

B. Li, Cold Spring Harbor Laboratory, New York: The synaptic circuitry of lateral habenula and learned helplessness.

R. Malinow, University of California, San Diego: The synaptic and cellular changes in the lateral habenula of learned helplessness in rats.

G. Goelman, Hadassah Hebrew University Medical Center, Jerusalem, Israel: Role of the habenula in depression that accompanies Parkinson's disease.

J. Watson, R. Malinow

B. Li, S. Sesack

Epigenetic Reprogramming and Transgenerational Inheritance

March 7–10

It is becoming clear that in mammals, plants, and other organisms, epigenetic mechanisms interface with genetic ones in regulating developmental decisions and pathways. Epigenetic reprogramming in the germ line and in early embryos allows pluripotency and stem cell plasticity, whereas the restriction of developmental plasticity also involves epigenetic mechanisms. Erasure of epigenetic marks in the germ line may be incomplete, leading to epigenetic inheritance of altered developmental potential across generations. In plants, there is growing evidence for a parallel reprogramming event in both the male and the female germ lines. In pollen, this results in transposon activation followed by small RNA production and transport into gametes. This represents a mechanism for reinforcement of transposon control in each generation.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **W. Reik,** Babraham Institute, Cambridge, United Kingdom

SESSION 1: Developmental Decisions
Chairperson: M.A. Surani, University of Cambridge, United Kingdom

T. Hiiragi, Max-Planck Institut für Molekulare Biomedizin, Muenster, Germany: Stochastic patterning in the development of pluripotency in mouse embryo.

M.-E. Torres-Padilla, Institute de Génétique et de Biologie, Strasbourg, France: Histone variants establish specialized chromatin signatures during reprogramming.

K. Hochedlinger, Harvard Stem Cell Institute, Cambridge, Massachusetts: Epigenetic similarities and differences between ES cells and iPS cells.

E. Heard, Institut Curie, Paris, France: Lessons from in vivo studies on X-chromosome inactivation and reactivation in different animals.

H. Blau, Stanford University School of Medicine, California: Role of active DNA demethylation by AID in reprogramming toward pluripotency.

P. Avner, Institut Pasteur, Paris, France: Developmental regulation of X-chromosome inactivation.

SESSION 2: Reprogramming and Reproduction
Chairperson: E. Heard, Institut Curie, Paris, France

R. Martienssen, Cold Spring Harbor Laboratory, New York: Heterochromatin reprogramming and small RNA silencing in plant germ cells.

F. Berger, National University of Singapore: Reprogramming histone modifications in *Arabidopsis.*

J. Walter, Saarland University, Saarbrücken, Germany: Epigenetic reprogramming in the mouse zygote: Lessons from bisulphate sequencing.

M. Surani, University of Cambridge, United Kingdom: Resetting the epigenome in embryos and germ line in the mouse.

R. Fischer, University of California, Berkeley: Reprogramming the endosperm genome.

W. Reik, Babraham Institute, Cambridge, United Kingdom: Reprogramming and programming of DNA methylation patterns in the genome.

SESSION 3: Imprints and Inheritance
Chairperson: T. Bestor, Columbia University, New York, New York

H. Sasaki, Medical Institute of Bioregulation, Kyushu, Fukuoka, Japan: Role of the PIWI pathway in DNA methylation of the imprinted *Rasgrf 1* locus in the male mouse germline.

A. Ferguson-Smith, University of Cambridge, United Kingdom: Genomic imprinting and the stability of the epigenetic program in developmental processes.

V. Colot, Ecole Normale Superieure, Paris, France: Transgenerational inheritance of epigenetic variation in *Arabidopsis*: Mechanisms and phenotypic impact.

D. Bourc'his, Institut Curie, Paris, France: Dnmt3L-independent way to methylate mammalian genomes.

W. Kelly, Emory University, Atlanta, Georgia: Epigenetic inheritance and reprogramming through the germline in *C. elegans.*

J. Paszkowski, University of Geneva, Switzerland: Stability of transgenerational epigenetic inheritance.

SESSION 4: Environmental Effects and Evolution
Chairperson: J. Rafalski, Dupont Experimental Station, Wilmington, Delaware

N. Heintz, The Rockefeller University, New York, New York: The significance of 5-hydroxymethylctytosine in neuronal function.

A. Rao, Harvard Medical School, Boston, Massachusetts: Biological functions of TET proteins, enzymes that convert 5 methylcytosine to hydroxymethylcytosine DNA.

D. Ruden, Wayne State University, Detroit, Michigan: Epigenomic reprogramming of aggression in killer bees.

J. Finnegan, CSIRO, Black Mountain, Australia: Flicking the switch on polycomb-regulated genes.

B. Hohn, Friedrich Miescher Institute of Biomedical Research, Basel, Switzerland: Environmental influences on plant genome dynamics.

U. Grossniklaus, Institute of Plant Biology, University of Zurich, Switzerland: Role of epigenetic regulation in evolution: The control of pollination syndromes.

SESSION 5: Methylation Mechanisms
Chairperson: H. Sasaki, Medical Institute of Bioregulation, Kyushu, Higashiaku, Japan

T. Bestor, Columbia University, New York, New York: How much tissue-specific DNA methylation and how important?

Y. Zhang, HHMI, University of North Carolina, Chapel Hill: Could the DNA demethylase please stand up?

S. Jacobsen, University of California, Los Angeles: DNA methylation in *Arabidopsis.*

General Discussion: W. Reik, Babraham Institute, Cambridge, United Kingdom

Concluding Remarks: R. Martienssen, Cold Spring Harbor Laboratory, New York

H. Blau, Y. Lazebnik

The Second NIMH-Sponsored Brain Camp

March 13–16

FUNDED BY   **National Institute of Mental Health**

ARRANGED BY   **M. Akil,** National Institute of Mental Health, Bethesda, Maryland
                T. Insel, National Institute of Mental Health, Bethesda, Maryland

We were very happy that the NIMH Brain Camp returned to Banbury in 2010 after its successful inauguration in 2009. 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 contributed to the meeting. The outcome of the meeting will be the start of a neuroscience curriculum that can eventually be shared with psychiatry training programs around the country.

Introduction and Charge: **M. Akil,** National Institute of Mental Health, Bethesda, Maryland
Rethinking Mental Illness: **T. Insel,** National Institute of Mental Health, Bethesda, Maryland

SESSION 1: Circuitry Underlying Aggression/Circuitry of Emotional Learning
R. Yuste, Columbia University, New York, New York
D. Salzman, Columbia University, New York, New York

SESSION 2: How Circuits Develop
C. Nelson, Harvard University, Boston, Massachusetts
B.J. Casey, Cornell University, Ithaca, New York

Discussion with the Organizers: Teaching neuroscience in medical school and during psychiatry training: What's missing?

SESSION 3: Stress from Molecules to Circuits
H. Akil, University of Michigan, Ann Arbor

SESSION 4: Circuitry Underlying Fear, Anxiety, and Recovery
J. LeDoux, New York University, New York, New York
E. Phelps, New York University, New York, New York

Roundtable Discussion with All Speakers

SESSION 5: Translation: From Neurobiology to Treatments
K. Berman, National Institute of Mental Health, Bethesda, Maryland
M. Bear, Massachusetts Institute of Technology, Cambridge

377

How Can We Maintain the Stability of Biomedical Research and Development at the End of the ARRA?

The American Recovery and Reinvestment Act (ARRA) has provided an extraordinary $8.2 billion in extramural funding to the National Institutes of Health and $3 billion to the National Science Foundation as well as sizable funds to other agencies. These funds have been used to promote research, construct and renovate buildings and facilities, and purchase shared instrumentation. Biomedical research has benefited greatly from the ARRA, but there are increasing concerns about what will happen when the program comes to an end. It is important that the young researchers whose careers have been promoted through the support of the stimulus funds are not abandoned and that promising projects initiated using ARRA funds are not delayed. This workshop was held to discuss ways in which the government, universities, and other groups can "smooth" spending over time and find other ways to avoid problems.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introduction: **ARRA and NIH, and Government Support for Science**
                         R. Freeman and **A. Wang,** NBER Science and Engineering Workforce Project, Harvard University, Cambridge, Massachusetts

SESSION 1: How NIH Spent the ARRA Funding and Thoughts About Post-ARRA
Points to Consider
L. Tabak, National Institute of Dental and Craniofacial Research, Bethesda, Maryland
J. Niederhuber, National Cancer Institute, Bethesda, Maryland
P. Stephan, Andrew Young School of Policy Studies, Georgia State University, Atlanta

SESSION 2: What Is the Hard-Landing Scenario for Research Careers? What Is the Best-Case Scenario?
Points to Consider
M. Teitelbaum, The Alfred P. Sloan Foundation, New York, New York
J. Wiest, National Cancer Institute, Bethesda, Maryland
H. Garrison, Federation of American Societies for Experimental Biology, Bethesda, Maryland

SESSION 3: How Can Federal Agencies Manage the End of ARRA?
Points to Consider
S. Turner, University of Virginia, Charlottesville
J. McGowan, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland
D. Mowery, University of California, Berkeley

SESSION 4: How Can Universities and Research Institutes Manage the End of ARRA?
Points to Consider
D. Korn, Harvard University, Cambridge, Massachusetts
B. Stillman, Cold Spring Harbor Laboratory, New York

SESSION 5: How Can PIs Manage the End of ARRA?
Points to Consider
G. Marschke, National Bureau of Economic Research, Cambridge, Massachusetts
R. Kolter, Harvard Medical School, Boston, Massachusetts
M. Carlson, Howard Hughes Medical Institute, Chevy Chase, Maryland
F. Murray, MIT Sloan School, Cambridge, Massachusetts

SESSION 6: Innovative Approaches to Managing Support for Science
Points to Consider
W. Goldschmidts, Cold Spring Harbor Laboratory, New York
D. Goroff, The Alfred P. Sloan Foundation, New York, New York
W. Schaffer, National Institutes of Health, Bethesda, Maryland

Summary and Concluding Remarks
R. Freeman, NBER Science and Engineering Workforce Project, Harvard University, Cambridge, Massachusetts
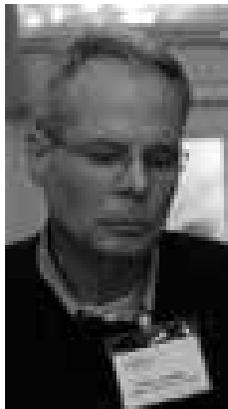
W. Schaffer

G. Marschke, P. Stephan

R. Freeman

Linguistic Phenotypes: Toward a Biological Understanding of Language

The general idea of this meeting was that language provides a window into mental function that can be exploited for the study and understanding of neuropsychiatric disorders. One of the outstanding problems in the study of neuropsychiatric disorders is the relative paucity of objectively defined phenotypic measures, which can be used for diagnoses and in psychiatric genetic studies. Linguistic performance reflects thought processes, so the hypothesis is that quantitative measures defined on the basis of linguistic performance could be used to characterize phenotypic variability among individuals. Autism (which includes communication disorders), specific language impairment, as well as Williams syndrome (where language seems to be a relative strength) are of particular interest in this regard. Schizophrenia (which includes thought disorder in its symptom list) may also be amenable to useful linguistic phenotyping. Participants included linguists and psycholinguists interested in human neurobiology and disease, as well as biologists working on autism, schizophrenia, and other neuropsychiatric disorders.

A major goal of the meeting was to bring together scientists who are doing linguistic phenotyping with experts in the syndromes and geneticists, in an attempt to push along what we hope will be an exciting field that can make major contributions to one of the most important scientific (theoretical and applied) topics concerning humans.

Introductory Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York

INTRODUCTORY SESSION
Chairperson: R. Berwick, Massachusetts Institute of Technology, Cambridge

P. Mitra, Cold Spring Harbor Laboratory, New York:
  Introduction.
N. Chomsky, Massachusetts Institute of Technology,
  Cambridge: Language as a biological organ: What is it?
  How does it develop? And why?
K. Wexler, Massachusetts Institute of Technology, Cambridge:
  Optional infinitives: A worked-out model of a particular
  linguistic characterization/phenotype.

S. Fisher, University of Oxford, United Kingdom: Building
  bridges between ge
nes, brains, and language.
A. Monaco, Wellcome Trust Centre for Human
  Genetics, Oxford, United Kingdom: Genetics of specific
  language impairment.

SESSION 1
Chairperson: A.-M. Di Sciullo, Université du Québec à Montréal, Canada

N. Modyanova and K. Wexler, Massachusetts Institute of
  Technology, Cambridge: Linguistic phenotypes of more
  complex syntax: Phases and determiners in SLI, Williams
  syndrome, and autism.
A. Perovic, University College London, United Kingdom:
  Grammatical impairments in autism spectrum, Williams
  syndrome, and Down syndrome.

L. Kovelman, University of Michigan, Ann Arbor: Optional
  infinitive: Evidence of how adult brain processes grammatical
  errors that are typical.

SESSION 2:
Chairperson: K. Wexler, Massachusetts Institute of Technology, Cambridge, Massachusetts

L.-A. Petitto, University of Toronto, Canada: The phonetic,
  genetic, and brain-based changes that give rise to early
  language acquisition. Genes, brains, and cognition:
  space and language in Williams syndrome.
M.-T. Guasti, University of Milan-Biocca, Milan, Italy: SLI
  and dyslexia: Differences between linguistic disorders in
  children.

General Discussion
  B. Landau, Johns Hopkins University School of Medicine,
  Baltimore, Maryland
  K. Wexler, Massachusetts Institute of Technology, Cambridge
  P. Mitra, Cold Spring Harbor Laboratory, New York

Moderated Discussion: White Paper

SESSION 3
Chairperson: P. Mitra, Cold Spring Harbor Laboratory, New York

D. Kimbrough Oller, University of Memphis, Tennessee and
  P. Niyogi, University of Chicago, Illinois: Vocal development
  theory and the automated identification disorders including
  autism.
P. Suppes, Stanford University, California: Brain
  representations of linguistic constituents.
P. Freed, Columbia University, New York, New York:
  Applications to clinical psychiatric practice.

N. Schiff, Weill Cornell Medical College, New York, New
  York: Recovery of consciousness after severe brain injury.
  Opportunities for linguistics to inform research.
D. Fox, Massachusetts Institute of Technology, Cambridge:
  Logic, language, and modularity.

SESSION 4
Chairperson: N. Modyanova, Massachusetts Institute of Technology, Cambridge

L. Osborne, University of Toronto, Canada: Duplication of
  genes on human chromosome 7q11.23 and their role in
  speech and expressive language.
C. Mervis, University of Louisville, Kentucky: Speech and
  language abilities of individuals with Williams syndrome.

S. Desiderio, Johns Hopkins School of Medicine, Baltimore,
  Maryland: TFII-I, a target of genetic lesions associated with
  the Williams–Beuren cognitive profile.
W. McCombie, Cold Spring Harbor Laboratory, New York:
  Genetics becomes genomics.

Discussion: Future Planning

Tumor Microenvironment and Metastasis

Approximately 80% of deaths related to cancer are associated with metastasis, and the central unanswered question remains the mechanism behind the systemic spread of cancer and secondary tumor formation in distant organs. It is clear that the microenvironment of a cancer cell has a key role in influencing its behavior and that this environment includes other cells and the tumor-associated extracellular matrix. The question, then, is what role does the tumor microenvironment have in metastasis? Is all the information needed for a cell to metastasize cell-autonomous or does the tissue microenvironment have a role in determining this process? Does disrupting the tumor microenvironment have a positive or negative impact on metastatic potential of cancer cells? Can the microenvironment explain why many primary tumors favor secondary tumor formation in specific organs? The meeting explored what constitutes the tumor microenvironment and examined its functional impact on metastasis with specific focus on new targets for treatment of metastatic cancer.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **J. Watson,** Cold Spring Harbor Laboratory, New York

SESSION 1
Chairperson: R. Kalluri, Harvard Medical School, Boston, Massachusetts

J. Massague, Memorial Sloan-Kettering Cancer Center, New York, New York: Surviving the microenvironment.
Z. Werb, University of California, San Francisco: Transcriptional regulation of metastasis.

SESSION 2
Chairperson: Z. Werb, University of California, San Francisco

H. Dvorak, Beth Israel Deaconess Medical Center, Boston
  Massachusetts: Angiogenesis: The wrong therapeutic target?
D. McDonald, University of California, San Francisco:
  Angiogenesis inhibitors: Risk and return.
A. Harris, The Weatherall Institute of Molecular Medicine,
  Oxford, United Kingdom: Role of notch signaling and anti-
  VEGF therapy resistance.
H. Hurwitz, Duke University Medical Center, Durham, North
  Carolina: Anti-angiogenesis therapy for cancer-expected and
  unexpected outcomes.

SESSION 3
Chairperson: K. Cichowski, Brigham & Women's Hospital, Boston, Massachusetts

M. Clarke, Stanford University, Palo Alto, California:
  Regulation of self-renewal by the microenvironment in
  normal epithelial stem cells and epithelial cancer cells or stem
  cells and cancer, two faces.
R. Bjerkvig, University of Bergen, Norway: Cancer stem cells:
  Can they be defined?
S. Haggarty, Massachusetts General Hospital, Boston: Patient-
  specific stem cell modes for characterizing disease and
  therapeutic discovery.
J. Chang, Baylor College of Medicine, Houston, Texas: Multi-
  targeting of key self-renewal pathways.

SESSION 4
Chairperson: J. Massague, Memorial Sloan-Kettering Cancer Center, New York, New York

R. Kalluri, Harvard Medical School, Boston, Massachusetts:
  Pericyte coverage of tumor vessels: An adaptive host response to
  control tumor hypoxia?
J. Sleeman, University of Heidelberg, Germany: The
  significance of lymphatic dissemination for metastasis: Blind
  alley, highway, or beacon?
K. Cichowski, Brigham & Women's Hospital, Boston,
  Massachusetts: The inflammation, the microenvironment,
  and prostate cancer metastasis.
D. Cheresh, University of California, San Diego, La Jolla:
  microRNA-132 mediated loss of p120RasGAP activates
  quiescent endothelium to facilitate pathological angiogenesis
  and tumor growth.

SESSION 5
Chairperson: S. Mohla, National Cancer Institute, Bethesda, Maryland

S. Rafii, Weill Cornell Medical Center, New York, New York:
  Contribution of the activated vascular niche to tumor growth.
D. Lyden, Weill Cornell Medical Center, New York, New
  York: Early cellular molecular events for the information of
  metastatic niche.
R. Hynes, Massachusetts Institute of Technology, Cambridge:
  Extracellular matrices of tumors.

SESSION 6
Chairperson: Y. Kang, Princeton University, New Jersey

S. Dias, Portuguese Institute of Oncology, Lisbon, Portugal:
  Metabolism and metastasis.
S. Muthuswamy, Ontario Cancer Institute, University of
  Toronto, Canada: Cell polarity and cancer progression.
O. Casanovas, Catalan Institute of Oncology, Barcelona,
  Spain: Tumor-adaptive responses to antiangiogenic therapies.
E. Sahai, Cancer Research UK, London, United Kingdom:
  Cancer cell invasion in a complex environment.
R. Sordella, Cold Spring Harbor Laboratory, New York:
  Intrinsic and extrinsic regulation of metastatic spread of
  NSCLC.

SESSION 7
Chairperson: D. Lyden, Weill Cornell Medical College, New York

M. Skobe, Mount Sinai School of Medicine, New York, New
  York: Role of lymphangiogenesis in tumor metastasis.
J. Condeelis, Albert Einstein College of Medicine, Bronx, New
  York: Imaging of the tumor microenvironment of metastasis
  and the cell types within.
Y. Kang, Princeton University, New Jersey: Tumor–stromal
  interactions in bone metastasis: Novel targets for
  therapeutic invention.

Genetic Variation at a Single Locus for Prediction and Prevention of Late-Onset Alzheimer's Disease

Heterogeneity of response of individuals selected for clinical trials is a serious problem. Participants in this workshop explored ways to incorporate "pharmacogenetics" as a tool in the selection of individuals with pre-Alzheimer's disease for anti-Aβ peptide immunotherapy. There was also discussion of how sequence variation is used to develop genome-wide association maps for determining how genomes are organized and regulated and their role in disease.

Opening Remarks: **J. Watson,** Cold Spring Harbor Laboratory, New York
                        C. Wang, United Biomedical, Inc., Hauppauge, New York

SESSION 1: Genetic Variation and Early Detection of Alzheimer's Disease

A. Roses, Deane Drug Discovery Institute, Duke University and Cabernet Pharmaceuticals, Durham, North Carolina: Tomm40 variable-length polymorphism predicts the age of late-onset Alzheimer's disease (LOAD).

T. Gingeras, Cold Spring Harbor Laboratory, New York: Origin of phenotypes: Genes and transcripts.

SESSION 2: Immunotherapy and Immunoprevention of Alzheimer's Disease

M. Weksler, Weill Cornell Medical College, New York, New York: Role of a segmental immunodeficiency in age-associated and trisomy 21-associated dementia: From immunotherapy to immunoprevention.

N. Relkin, Weill Cornell Medical College, New York, New York: Brain-imaging studies used in a phase II clinical study of intravenous immunoglobulin (IVIg) in patients with Alzheimer's disease.

P. Szabo, Weill Cornell Medical College, New York, New York: Amyloid-β peptide targets for immunotherapy of Alzheimer's disease.

C. Finstad, A. Walfield, F. Lin, K. Zhao, M.-J. Liao, C. Hung, C. Wang, United Biomedical, Inc., Hauppauge, New York and S. Lynn, B. Kuo, S.-N. Hsu, United Biomedical, Inc., Asia, Taiwan, Republic of China: Active immunization with UBITh® amyloid-β1-14 synthetic peptide vaccine (UB311): Design, development, and phase I clinical study.

SESSION 3: Discussion and Summary

A. Roses, D. Crenshaw, D. Burns, Duke University School of Medicine, Durham, North Carolina and I. Grossman, T.W. Swanson, Cabernet Pharmaceuticals, Durham, North Carolina: Use of "Pharmacogenetics" in study design as a

tool to maximize the benefit/risk profile of a compound in development, particularly at "proof of concept." Suitable biomarkers for early detection of Alzheimer's disease and clinical trial design.

Discussion Topics
- Phase II clinical trial protocol for UBITh® Aβ1-14 vaccine (UB311) in individuals with mild Alzheimer's disease
- Phase II clinical trial protocol for UBITh® Aβ1-14 vaccine (UB311) in individuals with mild cognitive impairment (MCI) selected through MCI enrichment screening (amnesia testing and FDG-PET scan) with posthoc genotyping of APOE and 523 locus in TOMM40
- Retrospective genotyping of APOE and 523 locus in TOMM40 in normal and individuals with Alzheimer's disease in a Chinese population
- Experience with FDG-PET and PiB-PET for early diagnosis of individuals with pre-Alzheimer's disease

Robertson House patio

Mutagenesis: What It Means and How It Has Changed

Mutation is, of course, an integral part of genetics, although what are considered mutations has changed over the years as new techniques have led to new knowledge, leading to revisions of what "mutation" encompasses. This discussion meeting reviewed the developing concepts of mutation and understanding of the varied forms of mutation from the early days of *Drosophila* genetics to the present. Although the theme of the meeting was the history of the concept of mutation, contemporary research was also included, for example, on copy-number variation.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **J. Watson,** Cold Spring Harbor Laboratory, New York

SESSION 1

S. Mueller-Wille, University of Exeter, United Kingdom: The taxonomic roots of mutation: Constant varieties, sports, and pure lines.

E. Carlson, Bloomington, Indiana: Mutation and the gene in H.J. Muller's course, career, and influence.

S. Brenner, Salk Institute for Biological Studies, San Diego, California: Discovery of frame-shift mutants.

R. Falk, Hebrew University of Jerusalem, Israel: Mutagenesis as a genetic research strategy.

L. Campos, Drew University, Madison, New Jersey: From experimental evolution to genetic engineering: Mutation at Cold Spring Harbor Laboratory.

W. Maas, New York University School of Medicine, New York, New York: Role of serendipitous mutants in the elucidation of gene action and its regulation.

Who Are They? Identifying Participants in the Cold Spring Harbor Laboratory Symposia Photographs

SESSION 2

B. Bridges, Romsey, United Kingdom: From phenomenology to molecular understanding: Early work with ionizing radiation mutation.

A. Creager, Princeton University, New Jersey: Mutation in the atomic age.

B. Ames, Children's Hospital Oakland Research Institute, California: Mutation, detecting mutagens, and cancer prevention.

J. Drake, National Institute of Environmental Health, Research Triangle Park, North Carolina: Artifacts of mutation ascertainment.

J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York: Cold Spring Harbor Laboratory's contribution to the Human Genome Project.

L. Pollock, Cold Spring Harbor Laboratory, New York: International data repository for the history of the Human Genome Project.

B. Ames, Children's Hospital Oakland Research Institute, California: Delaying cancer and other age-related diseases with micronutrients.

N. Fedoroff, U.S. Department of State, Washington, D.C.: McClintock, mutations, and epimutations.

E. Friedberg, University of Texas Southwestern Medical School, Dallas: The molecular mechanism of DNA-damage-induced mutagenesis: After the SOS phenomenon to the present.

Who Are They? Identifying Participants in the Cold Spring Harbor Laboratory Symposia Photographs

SESSION 3

P. Hanawalt, Stanford University, California: Role of transcription in mutagenesis and genomic stability.

M. Lynch, Indiana University, Bloomington: Evolution of the mutation rate.

G. Montgomery, New York, New York: Changing views on sequence and synthesis.

G. Walker, Massachusetts Institute of Technology, Cambridge: Positively-acting functions required for mutagenesis: Function and control of translesion DNA polymerases.

C. Weissmann, Scripps Research Institute, Jupiter, Florida: Mutation-like events during prion propagation.

W. Maas, R. Maas, B. Ames

S. Brenner, J. Witkowski

E. Friedberg, E. Witkin

PopTech Science and Public Leadership Fellows Retreat

August 11–15

FUNDED BY   **PopTech Accelerator**

ARRANGED BY   **L. Filderman,** PopTech, Camden, Maine
                   O. Wilder, PopTech, Camden, Maine

The PopTech Science and Public Leadership Fellows Program aims to develop a corps of extremely high potential, socially engaged working scientists who embody science as an essential way of thinking, discovering, understanding, and deciding, and who can communicate both their work and the importance of their fields to the public at large. The program gives these scientists intensive, high-quality training, a powerful social network, ongoing mentoring, and opportunities for public leadership and engagement. The Banbury Center was delighted to host the Fellows for the first meeting of the science and public leadership group.

Welcome and Introductions: **J. Witkowski,** Executive Director, Banbury Center, Cold Spring Harbor Laboratory, New York
A. Zolli, Executive Director and Curator, PopTech, Camden, Maine

C. Heller, CEO, Heller Communication Design, Brooklyn, New York: Communicating for engagement.

M. Moon, Communication Designer, New York, New York: Art of the great presentation.

S. Marsh, Theatre Arts, Stony Brook University, New York: Improv for scientists—Robertson House.

M. Duenwald, Deputy Editor, Op-Ed Page, *The New York Times*, New York, New York: Demystifying the Op-Ed page.

L. Witter, Chief Strategy Officer, Fenton, New York: Media training.

H. Schneider, Dean, School of Journalism, Stony Brook University, New York: Communicating science in a changing media landscape.

Panel Discussion: The Journalists' Perspective
  L. Cuthbert, Director, *Discovery News*, New York, New York

B. Nissen, Senior Producer, NBC News-NBC Learn, New York.

M. Moyer, Senior Technology Editor *Scientific American*, New York, New York and I. Oransky, Executive Editor, Reuters Health: *Science*, New York, New York: The journalist's perspective.

M. Nisbet, Associate Professor, School of Communication, American University, Washington, D.C. and R. Covey, Senior Vice President, National Geographic Digital Media, Washington, D.C.: Framing scientific issues to foster engagement.

G. MacKenzie, Founder and President, Gillian MacKenzie Agency, New York, New York; W. Murphy, Senior Editor, Random House, New York, New York; T. Neillsen, CEO, BrightSight Group, Princeton, New Jersey: Creating a personal brand.

Plant Development and Phenomics

September 19–22

FUNDED BY   **Cold Spring Harbor Laboratory/Pioneer–DuPont Joint Collaborative Research Program**

ARRANGED BY   **D. Jackson,** Cold Spring Harbor Laboratory, New York
                      H. Sakai, Pioneer–DuPont International, Johnston, Texas

Every year, the Cold Spring Harbor Laboratory–DuPont Pioneer Collaborative meets to review current projects and to plan future projects. This year's update focused on the area of phenomics and its relation to plant development and included current research from outside the collaboration by speakers working in this field.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **D. Jackson,** Cold Spring Harbor Laboratory, New York
                            S. Tingey, DuPont Experimental Station, Wilmington, Delaware

SESSION 1: Informatics, Natural Variation, and Yield
Chairperson: Z. Lippman, Cold Spring Harbor Laboratory, New York

W. McCombie, Cold Spring Harbor Laboratory, New York: Novel sequencing methodologies and results.

B. Fendler, Cold Spring Harbor Laboratory, New York: Population genetics of microsatellites in humans.

D. Ware, Cold Spring Harbor Laboratory, New York: Profiling maize using next-generation sequencing approaches.

Z. Lippman, Cold Spring Harbor Laboratory, New York: Flowering, dosage, and yield.

R. Lafitte, Pioneer Hi-Bred International, Johnston, Iowa: Maize field phenotyping for transgene validation.

M. Williams, Dupont Experimental Station, Wilmington, Delaware: Identifying maternal haploid-inducing QTL1 and ENU-mutagenesis of maize.

SESSION 2: Development, Epigenetics, and Networks
Chairperson: M. Aukerman, Dupont Experimental Station, Wilmington, Delaware

P. Bommert, Cold Spring Harbor Laboratory, New York: Cloning of compact Plant2 and update on other fasciated mutants.

M. Komatsu, DuPont Experimental Station, Wilmington, Delaware: Inflorescence traits and hybrid production.

M. Aukerman, Dupont Experimental Station, Wilmington, Delaware: A putative silencing suppressor identified from an NUE screen.

K. Creasey, Cold Spring Harbor Laboratory, New York: Developmental interaction of DDM1 and RNAi.

J. Han, Cold Spring Harbor Laboratory, New York: Identification of high-copy mutator insertions in MTM lines by Illumina sequencing.

A. Eveland, Cold Spring Harbor Laboratory, New York: Expression profiling of maize inflorescences.

C. MacAlister, Cold Spring Harbor Laboratory, New York: Control of meristem size and identity at the transition to flowering in tomato: Role of terminating flower and fasciated flower.

S. Kumari, Cold Spring Harbor Laboratory, New York: Genome-wide computational predications of core promoter elements in plant genomes.

SESSION 3: Phenomics and Shoot Development
Chairperson: R. Williams, DuPont Experimental Station, Wilmington, Delaware

E. Spalding, University of Wisconsin, Madison: Root phenomics.

T. Altmann, Leibniz Institute of Plant Genetics and Crop, Gatersleben, Germany: Analysis of *Arabidopsis* natural genetic variation and heterosis in biomass accumulation and metabolism.

G. Li, Pioneer Hi-Bred International, Johnston, Iowa: HTP greenhouse phenotyping: Challenges and promises.

Y. Eshed, Weizmann Institute of Science, Rehovot, Israel: Leaf development.

M. Scanlon, Cornell University, Ithaca, New York: Maize shoot apical meristem genomics.

SESSION 4: Reproductive and Root Development
Chairperson: D. Jackson, Cold Spring Harbor Laboratory, New York

J. Kyozuka, Tokyo University, Japan: Rice inflorescence development.

U. Grossniklaus, Institute of Plant Biology University of Zurich, Switzerland: Genetic approaches toward the engineering of apomixis.

D. Jackson, Cold Spring Harbor Laboratory, New York: Maize inflorescence development.

G. Taramino, DuPont AgBiotechnology, Johnston, Iowa: Toward understanding the genetic network controlling maize root architecture.

R. Martienssen, Cold Spring Harbor Laboratory, New York: Heterochromatin reprogramming and germ cell fate.

S. Brady, University of California, Davis: Root networks.

SESSION 5: Epigenetics and General Discussion
Chairperson: R. Martienssen, Cold Spring Harbor Laboratory, New York

A. Rafalski, Dupont Experimental Station, Wilmington, Delaware: Update on epigenotyping method development at Pioneer/Dupont.

M. Regulski, Cold Spring Harbor Laboratory, New York: Epigenetic project.

J. Lu, Cold Spring Harbor Laboratory, New York: Epigenetic project.

M. Dotto, Cold Spring Harbor Laboratory, New York: AGO10-associated small RNAs and tasiRNA pathways in maize.

K. Petsch, Cold Spring Harbor Laboratory, New York: Genetic dissection of inbred-specific modifiers of the maize tasiRNA pathway.
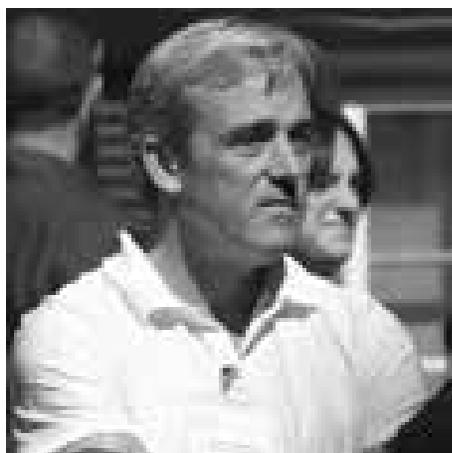
S. Tingey

E. Spalding, S. Brady

Fragile X Syndrome: Current Status, Future Prospects

The Banbury meetings on Fragile X syndrome have served as a major catalyst for the rapid advances the field has made over the last decade. These include elucidation of the molecular functions of FMRP; formation of the mGluR theory of Fragile X; expanded descriptions of behavioral phenotypes present in humans and animal models; identification of FMRP targets that have led to novel pathways for potential pharmacotherapy; and to several clinical trials. The present meeting was devoted to a critical review of where the field stands, an examination of the current debates about mechanisms and treatments, and determination of the essential next steps.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Fragile X Portraits: **K. Clapp,** FRAXA Research Foundation, Newburyport, Massachusetts

SESSION 1: Clinical Trials and Outcome Measures
Chairperson: D. Nelson, Baylor College of Medicine, Houston, Texas

M. Tranfaglia, FRAXA Research Foundation, Newburyport, Massachusetts: Psychopharmacology of Fragile X: Past, present, and future.

R. Hagerman, University of California Davis Health System, Sacramento: Arbaclofen trial: Seaside study.

S. Webb, University of Washington, Seattle: Can EEG be used to assess response to medication?

E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois: New outcome measures for clinical trials in FXS.

C. Erickson, Riley Hospital for Children, Indianapolis, Indiana: Commercially available glutamatergic agents in Fragile X syndrome: Pilot investigations.

SESSION 2: Synapses, Circuits, and Rhythms
Chairperson: K. Huber, University of Texas Southwestern Medical Center, Dallas

D. Rojas, University of Colorado, Denver School of Medicine: Gamma-band responses as potential biomarkers in autism and FXS.

W. Gan, New York University Medical Center, New York, New York: Abnormal experience-dependent dendritic spine plasticity in a mouse model of Fragile X.

A. Contractor, Northwestern University, Chicago, Illinois: Synapse development in the sensory cortex of FMR1 knockout mice.

M. Huntsman, Children's National Medical Center, Washington, D.C.: Inhibitory neurotransmission defects in Fragile X syndrome.

L. Kaczmarek, Yale University School of Medicine, New Haven, Connecticut: FMRP, ion channels, and the regulation of neuronal timing.

B. Alger, University of Maryland School of Medicine, Baltimore: Endocannabinoids and a mouse model of Fragile X syndrome.

O. Manzoni, Neurocentre Magendie, Bordeaux, France: Disorganization of the endocannabinoid perisynaptic signaling machinery in $FMR1^{-/-}$ mice.

R. Wong, SUNY–Downstate Health Science Center, Brooklyn, New York: Mechanism of mGLuR-mediated plasticity.

SESSION 3: Receptors to Ribosomes
Chairperson: P. Vanderklish, Scripps Research Institute, La Jolla, California

K. Huber, University of Texas Southwestern Medical Center, Dallas: Mechanisms of mGluR5 dysfunction in Fmrl KO mice.

F. Tassone, Mind Institute, Davis, California: Altered mTOR-dependent signaling and differential mGluR expression patterns in Fragile X syndrome.

S. Zukin, Albert Einstein College of Medicine, Bronx, New York: FMRP acts via PIKE to regulate mTOR signaling.

G. Bassell, Emory University, Atlanta, Georgia: FMRP and miRNAs: Partners for translation at the synapse.

SESSION 4: FMRP Isoforms and Their Manipulation in Brain
Chairperson: I.J. Weiler, University of Illinois, Urbana-Champaign

D. Venkitaramani, University of Illinois, Urbana-Champaign: FMRP isoforms and restoration of function. Spatial, temporal, and splice variations in FMRP function.

D. Nelson, Baylor College of Medicine, Houston, Texas: FMR1 and FXRs.

SESSION 5: Targets and Model Systems
Chairperson: G. Bassell, Emory University, Atlanta, Georgia

C. Westmark, Waisman Center, Madison, Wisconsin: Reversal of Fragile X phenotypes by manipulation of APP/Aβ levels.

R. Jope, University of Alabama, Birmingham: Therapeutic effects of GSK3 inhibitors in Fragile X mice.

B. Oostra, Erasmus University, Rotterdam, The Netherlands: Rescue of behavioral phenotype and neuronal protrusion morphology in FMR1 KO mice.

S. Haggarty, Massachusetts General Hospital, Boston: Using

patient-specific iPS cells for modeling pathogenesis and treatment of Fragile X syndrome.

P. Vanderklish, Scripps Research Institute, La Jolla, California: Strategies for new target discovery and evaluation. Identification and validation of new targets for the treatment of FXS.

General Discussion: F. Gasparini, Novartis Pharma AG, Basel, Switzerland

F. Tassone

D. Nelson

Genome-Era Pathology, Precision Diagnostics, and Preemptive Care: A Stakeholder Summit

October 13–15

Historically, the discipline of pathology has had a central role to detect, classify, and interpret cellular and molecular markers of disease to guide physicians in the care and management of their patients. In recent years, many high-throughput technologies have been developed and used in research to determine the molecular and genetic mechanisms underlying diseases, but the pathology community has not responded systematically to the challenges and opportunities provided by these technological innovations. The objective of this discussion workshop was to define these opportunities, identify challenges and barriers to success, and formulate a call to action that will keep pathology at the forefront of modern medical care.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1: The Issue and Objective
Welcome and Overview: J. Saffitz, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

Brief Background Presentations
J. Schamberg, College of American Pathologists, Waukesha, Wisconsin: Pathology today: Challenges.

E. Green, National Human Genome Research Institute, Bethesda, Maryland: Genome-era pathology.

393

J. Saffitz, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts: Genome-era pathology and preemptive care.

M. Boguski, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts
Discussion: Objectives of genome-era pathology
Objective: Define and refine primary objectives, open issues, and potential action items

SESSION 2: Factors for Success

J. Crawford, North Shore LIJ Laboratories, Lake Success, New York
Discussion: Obstacles and challenges
Objective: Identify and prioritize primary challenges and identify potential solutions

P. Tonellato, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts and R. Haspel, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

Discussion: Overcoming the obstacles and challenges.
Objective: Review current efforts designed to resolve obstacles.
Identify key stakeholders, approaches and methods to overcome barriers and funding options that may provide the resources and forums to act on solutions

SESSION 3: A Call to Action

J. Crawford, North Shore LIJ Laboratories, Lake Success, New York, P. Tonellato, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts, and M. Boguski, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts

Discussion: Summarize objectives, action items, and next steps
Objective: Summarize main points and final action items

J. Saffitz, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts
Discussion: The horizon view

S. Tomlins, F. Barr

W. Rosenkrans, R. Jeter, H. Halvorson

Stem Cells, Genetics, and RNA-Binding Proteins: Recent Advances in ALS Research and Drug Discovery

October 17–19

FUNDED BY   **The ALS Association Greater New York Chapter**

ARRANGED BY   **L. Bruijn,** The ALS Association, Washington, D.C.
                    T. Maniatis, Columbia University Medical Center, New York, New York
                    C. Svendsen, University of Wisconsin, Madison

During the past decade, significant progress has been made in understanding the mechanisms leading to ALS. The focus has shifted from a motor neuron centric view to a recognition that the neighboring cells and in particular the glia have an integral role in the disease process. The landscape for ALS is again changing, and this meeting brought together leaders in diverse fields of ALS genetics, RNA processing, stem cells, and model systems to discuss how to capitalize on the promising research advances in all these fields and make an impact on ALS discoveries. Progress in developing new in vitro and in vivo systems to better understand the disease and the development of new tools for drug discovery were key topics of discussion at the workshop. The workshop provided a unique opportunity for academic scientists, clinicians, and the industry to discuss how better to understand the role of the new genes in ALS and how this impacts drug discovery.

T. Maniatis

Introduction: **L. Bruijn,** The ALS Association, Washington, D.C.
Introductory Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1: Genetics of ALS
Chairpersons: C. Shaw, Guy's Hospital, London, United Kingdom and R. Brown, University of Massachusetts,
  North Worchester

Overview of FUS/TDP43 Genetics and Pathology
C. Shaw, Guy's Hospital, London, United Kingdom
R. Brown, University of Massachusetts, North Worchester

Emerging Technologies in Genetics: Session Discussion

J. Hardy, Reta Lila Weston Institute of Neurological Studies,
  University College London, United Kingdom
R. Myers, Hudson Alpha Institute for Biotechnology,
  Huntsville, Alabama

SESSION 2: Disease Mechanisms and ALS
Chairperson: A. Goldberg, Harvard Medical School, Boston, Massachusetts

Disease Mechanism and Lessons Learned from SODI
D. Cleveland, University of California, San Diego, La Jolla

**Protein Misfolding and Cell Death: Apoptosis, Autophagy,
  and Necrosis**
A. Goldberg, Harvard Medical School, Boston, Massachusetts

D. Borchelt, University of Florida, Gainesville

Glial–Neuronal Interactions and Inflammation
J. Rothstein, Johns Hopkins University School of Medicine,
  Baltimore, Maryland
B. Barres, Stanford University School of Medicine, California

SESSION 3: Animal Models
Chairperson: G. Cox, The Jackson Laboratory, Bar Harbor, Maine

R. Baloh, Washington University School of Medicine, St.
  Louis, Missouri
D. Cleveland, University of California, San Diego, La Jolla

P. Wong, Johns Hopkins University, Baltimore, Maryland
N. Shneider, Columbia University, New York, New York

SESSION 4: TDP43/FUS and Disease Mechanism
Chairperson: T. Maniatis, Columbia University Medical Center, New York, New York

V. Lee, University of Pennsylvania School of Medicine,
  Philadelphia

M. Moore, HHMI/University of Massachusetts Medical
  School, Worcester

SESSION 5: Stem Cells
Chairperson: C. Svendsen, Cedar Sinai Medical Center, Los Angeles, California

IPS Technology
F. Soldner, Whitehead Institute, Massachusetts Institute of
  Technology, Cambridge

IPS in ALS–Model Systems
C. Svendsen, Cedar Sinai Medical Center, Los Angeles,
  California
K. Eggan, Harvard University, Cambridge, Massachusetts

Assay Development Using Stem Cells
H. Wichterle, Columbia University, New York, New York
D. Fischer, BioFocus DPI, Leiden, The Netherlands
S. Finkbeiner, University of California, San Francisco

SESSION 6: Closing Session
Chairperson: C. Svendsen, Cedar Sinai Medical Center, Los
  Angeles, California

T. Maniatis, Columbia University Medical Center, New York,
  New York

J. Rothstein, D. Cleveland

The Calculus of Medicine: Treatment of Pancreatic Cancer as a Prime Exemplar

This meeting emphasized the importance of the integration of computer science, statistics, and mathematics into biomedical research and clinical trials, focusing on a better understanding of cancer, with pancreatic cancer as the prime exemplar. Key leaders in oncology research and clinical application discussed how to shape this exciting field (and community) with a particular focus on genomics, proteomics, imaging, translational bioinformatics, systems biology, disease modeling, single-cell analysis and nano-medicine.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **J.-P. Bizarri,** Celgene, Summit, New Jersey

SESSION 1: The Biology of Pancreatic Cancer
Chairperson: D. Tuveson, CRUK Cambridge Research Institute, United Kingdom

R. Hruban, Johns Hopkins Medical Institutions, Baltimore, Maryland: Genetics of pancreatic cancer.
D. Simeone, University of Michigan Health Systems, Ann Arbor: Pancreatic stem cells.

D. Bar-Sagi, New York University, New York, New York: Inter- and intra-cellular pathways in PDA.
D. Hedley, Ontario Cancer Institute, Toronto, Canada: Effects of the tumor microenvironment on invasion and metastasis.

D. Tuveson, CRUK Cambridge Research Institute, United Kingdom: Stromal barriers in pancreatic cancer.

M. Lisanti, Kimmel Cancer Center, Philadelphia, Pennsylvania: Warburg in reverse: The autophagic tumor stroma model of cancer.

M. Lotze, University of Pittsburgh, Pennsylvania: Autophagy as a target in pancreatic cancer.

SESSION 2: Clinical Developments in Pancreatic Cancer
Chairperson: M. Tempero, University of California, San Francisco

M. Tempero, University of California, San Francisco: New paradigms for drug development.

J. Berlin, Vanderbilt-Ingram Cancer Center, Nashville, Tennessee: New agents for treatment of pancreatic cancer.

M. Hidalgo, Centro National de Investigaciones Oncologicas, Madrid, Spain: Targeting the stroma in pancreatic cancer.

P. Philip, Karmanos Cancer Institute, Detroit, Michigan: Targeting IGF-IR in pancreatic cancer.

J. Clark, Massachusetts General Hospital, Boston: Modulating KRAS as a target in pancreatic cancer.

W. Isacoff, University of California, San Francisco: Novel chemotherapy schedules for pancreatic cancer.

SESSION 3: BioInformatics and BioMarkers in Pancreatic Cancer
Chairperson: B. Mishra, Courant Institute, New York University, New York, New York

C. Cantor, Sequenom Inc., San Diego, California: Cancer-specific nucleic acid sequences.

J. Reed, University of California, Los Angeles: Nano measurement approaches for characterizing single cells in populations.

B. Mishra, Courant Institute, New York University, New York, New York: Translational cancer bioinformatics.

J. Watson, L. Lisanti

D. Bar-Sagi, D. Simeone

Energy Metabolism, the Cell Cycle, and Cancer

In 1924, Otto Warburg drew attention to the fact that cancer cells generate energy largely through glycolysis and he believed that this was the fundamental characteristic of cancer cells. In recent years, there has been a renewal of interest in the Warburg effect, and this meeting focused on the general idea that proliferating cells may require more energy than quiescent cells and that energy production must therefore be coordinated with commitment to cell cycle entry. Specific topics included the Warburg effect; TOR and AKT signaling; metabolites controlling gene expression, energy metabolism, and the cell cycle; and effects of oxygen and reactive oxygen species. Although the meeting concentrated on mammalian systems, recent results from model systems such as yeast showing linkages between energy metabolism and cell cycle were also discussed.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **C. Van Dang,** Johns Hopkins Medical School, Baltimore, Maryland

SESSION 1: Glycolysis and Energy
Chairperson: J. Blenis, Harvard Medical School, Boston, Massachusetts

J. Chesney, University of Louisville, Kentucky: Fructose-2, 6-bisphosphate couples glycolysis with proliferation.

C. Van Dang, Johns Hopkins Medical School, Baltimore, Maryland: Oncogenic alterations of glucose and glutamine metabolism.

M. Vander Heiden, Massachusetts Institute of Technology, Cambridge: PKM2 and understanding the energetics of cancer cell metabolism.

R. Shaw, Salk Institute for Biological Studies, La Jolla, California: The LKB1/AMPK pathway: Tumor suppression and central regulators of metabolism.

M. Pollak, SMBD Jewish General Hospital, Montreal, Quebec, Canada: Host energy intake, cellular energy supply, and tumor growth: Roles of insulin and AMPK.

K. Struhl, Harvard Medical School, Boston, Massachusetts: Metformin selectively kills cancer stem cells and acts together with chemotherapy to prolong remission.

SESSION 2: Metabolic Regulation
Chairperson: J. Chesney, University of Louisville, Kentucky

Y. Xiong, University of North Carolina, Chapel Hill: Metabolic regulation in normal and tumor cells.

M. Lisanti, Kimmel Cancer Center, Philadelphia, Pennsylvania: Warburg in reverse: The autophagic tumor stroma model of cancer.

J. Brugge, Harvard Medical School, Boston, Massachusetts: Regulation of metabolism by extracellular matrix on oncogenes.

SESSION 3: PI3 Kinase, Isocitrate Dehydrogenase, TOR
Chairperson: L. Cantley, Beth Israel Deaconess Medical Center, Boston, Massachusetts

L. Cantley, Beth Israel Deaconess Medical Center, Boston, Massachusetts: PI3 kinase and cancer metabolism.

D. Schenkein, Agios Pharmaceuticals, Cambridge, Massachusetts: Mutations in isocitrate dehydrogenase: Role in disease pathogeneses and potential as a therapeutic target.

T. Mak, Campbell Family Institute for Breast Cancer Research at PMH, UHN, Toronto, Ontario, Canada: Knock-in mouse model for IDH1 mutations.

J. Blenis, Harvard Medical School, Boston, Massachusetts: Amino acids, mTOR signaling, and metabolism.

B. Manning, Harvard University, Boston, Massachusetts: mTOR activation drives the metabolic reprogramming of tumor cells.

M. Tyers, University of Edinburgh, United Kingdom: Metabolic and growth control by TOR effectors in yeast.

SESSION 4: Connections between Metabolism and Cell Cycle in Yeast
Chairperson: M. Tyers, University of Edinburgh, United Kingdom

B. Futcher, Stony Brook University, New York: Introductory remarks.

B. Tu, University of Texas Southwestern Medical Center, Dallas: Metabolic signals that drive cell growth and proliferation.

A. Caudy, Princeton University, New Jersey: The sedoheptulose bisphosphatase SHB17 shunts carbon from glycolysis to the pentose phosphate pathway for riboneo-genesis in yeast.

J. Broach, Princeton University, New Jersey: Direct control of metabolism by nutrient signaling pathways in yeast.

B. Futcher, Stony Brook University, New York: cAMP, liquidation of storage carbohydrates, and commitment to the cell cycle.

S. Kohlwein, Karl-Franzens-Universitat Graz, Austria: Lipid requirements during the cell cycle.

SESSION 5: Cancer Metabolism and p53
Chairperson: L. Cantley, Beth Israel Deaconess Medical Center, Boston, Massachusetts

E. White, Cancer Institute of New Jersey, New Brunswick: Role of autophagy in cancer metabolism.

K. Vousden, Beatson Institute for Cancer Research, Bearsden, Glasgow: The regulation of metabolism by p53.

A. Levine, Institute for Advanced Study, Princeton, New Jersey: Role of p53 in regulating metabolic pathways.

L. Cantley, Beth Israel Deaconess Medical Center, Boston, Massachusetts: Concluding remarks and general discussion.

J. Brugge

D. Schenkein
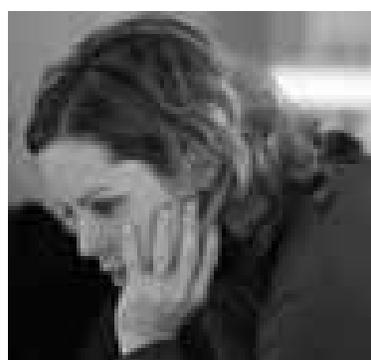
A. Caudy

Easeful Death: 21st Century Perspectives on Assisted Suicide

November 3–5

ORGANIZED BY   **M. Battin,** University of Utah, Salt Lake City
     E. MacDonald, Guy's and St. Thomas Hospital, London, United Kingdom
     T. Murray, The Hastings Center, Garrison, New York, New York
     M. Raff, University College London, United Kingdom
     J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

Because of advances in medical care, the proportion of the elderly in the population is increasing. And as a consequence, the number of individuals suffering from Alzheimer's, Parkinson's, and motor neuron diseases, as well as other age-related degenerative disorders, is increasing. Individuals suffering from these disorders place an extraordinary burden on their caregivers, who are often also elderly and who may be unable to cope. End-of-life issues involve profound legal, moral, religious, and biomedical questions and evoke such intense passions that calm discourse is hard to achieve. The Banbury Center provided a venue for calm discussions of topics, which included what are the data on assisted suicide and how (and why) do interpretations of the data differ? What is the evidence for abuse, what is its incidence and how might it be prevented? How can end-of-life decisions be made in cases of psychiatric illness (and perhaps dementia)? Participants included scientists, physicians, philosophers, lawyers, and religious leaders and included experts on euthanasia and assisted suicide in Europe.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **M. Raff,** University College London, United Kingdom

SESSION 1
Chairperson: T. Murray, The Hastings Center, Garrison, New York

M. Battin, University of Utah, Salt Lake City: Discussion.
B. Gert, Dartmouth College, Hanover, New Hampshire: Refusal of hydration and nutrition as an alternative to physician-assisted suicide.

D. Orentlicher, University of Iowa, Iowa City: Drawing lines at the end of death: Where can we draw meaningful lines among treatment withdrawal, assisted suicide, and euthanasia?

SESSION 2
Chairperson: E. MacDonald, Guy's and St. Thomas Hospital, London, United Kingdom

B. Onwuteaka-Philipsen, VU Medical Center, Amsterdam, The Netherlands: The influence of regulation on the practice of assisted suicide in The Netherlands.
L. Deliens, Vrije Universiteit, Brussels, Belgium: Belgian data on euthanasia.

L. Cohen, Baystate Medical Center, Northampton, Massachusetts: Accusations and investigations of euthanasia and PAS directed at American palliative medicine physicians.

Matters arising from Day 1

SESSION 3
Chairperson: R. Payne, Duke Divinity School, Durham, North Carolina

C. Baron, Boston College, Newton, Massachusetts: Law at the end of life: Have we come of age?
J. Lynn, Colorado Foundation for Medical Care, Chevy Chase, Maryland: Political realities and strategies: Improving care in the "death panel" era.

General Discussion: What is the 21st perspective on assisted suicide?

J. Prey, M. Raff

L. Deliens, T. Murray

Z. Schostak, B. Gert

Microbial Forensics in the Era of Genomics

November 7–10

FUNDED BY   **The U.S. Department of Homeland Security and individual participants**

ARRANGED BY   **B. Budowle,** University of North Texas Health Science Center, Fort Worth
T. Pals, U.S. Department of Homeland Security, Washington, D.C.
S. Schutzer, University of Medicine and Dentistry–New Jersey Medical School, Newark

This workshop brought experts in metagenomics together with experts from the field of microbial forensics and bioterrorism to consider the implications of whole-genome sequencing for investigations of bioterrorism. It is likely that whole-genome sequencing will become the method of choice to characterize a microbe and compare it with a reference sample. However, there are issues when considering strategic planning and implementation of genome-wide analyses for forensic attribution purposes. These include the development of reference databases for making inferences about the significance of an observation; the need to establish criteria to guide decision makers and scientists on performance and expectations; how to deal with degraded and trace evidence; how to improve analytical and sampling strategies for maximum extraction of information from large data sets; and cost.

Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1: Overview
Chairperson: S. Schutzer, University of Medicine and Dentistry–New Jersey Medical Center, Newark

B. Budowle, University of North Texas Health Science Center, Fort Worth: Microbial forensics in the era of genomics: Setting the stage.

R. Murch, Virginia Tech–National Capital Region, Alexandria: Overview of needs.

T. Pals, U.S. Department of Homeland Security, Washington, D.C.: Bioforensics basic research R&D program.

Discussion
Leaders:
   N. Bergman, National Biodefense Analysis and
   Countermeasures Center, Frederick, Maryland and A.

Phillippy, Battelle National Biodefense Institute, Frederick,
Maryland: Current analytical and bioinformatic tools
available to meet the national mandate for characterization of
microbial forensic evidence.

SESSION 2: Lessons from Case Studies
Chairperson: B. Budowle, University of North Texas Health Science Center, Fort Worth

R. Bull, Federal Bureau of Investigation, Frederick, Maryland:
   Lessons learned from anthrax attack and other cases.
D. Rock and W. Laegreid, College of Veterinary Medicine,
   Urbana, Illinois: Lessons not learned from animal agriculture
   cases.

T. Cebula, Johns Hopkins University, Baltimore, Maryland:
   Lessons learned from human food outbreaks.

SESSION 3: Technology: What Do We Currently Have and What Do We Need? If Money Is No Object;
   Philosophy/Fantasy
Chairperson: P. Pesenti, U.S. Department of Homeland Security, Washington, D.C.

P. Keim, Northern Arizona University, Flagstaff: Whole-
   genome sequencing, targeted genotyping, and the value of
   databases for investigation of plague infections.
W. Nierman, J. Craig Venter Institute, Rockville, Maryland:
   Forensic sequencing project accuracy with current sequencing
   production platforms and assembly and analysis tools.
J. Ravel, Institute of Genome Sciences, University of
   Maryland, Baltimore: Real-time or targeted marker typing
   capabilities.

C. Fraser-Liggett, University of Maryland School of Medicine,
   Baltimore: Current technologies and wish list.
W. McCombie, Cold Spring Harbor Laboratory, New York:
   Overview of bioinformatic capabilities.

B. Budowle, A. van Daal, J. Smith, S. Schutzer, T. Cebula

SESSION 4: Quality of Sample of Necessary Technology
Chairperson: J. Burans, U.S. Department of Homeland Security, Frederick, Maryland

M. Eshoo, Ibis Biosciences, Inc. Carlsbad, California:
  Extraction capabilities, DNA repair, and whole-genome
  amplification and analysis of trace unculturable specimens.

SESSION 5: Other Technologies Including Proteomics
Chairperson: S. Morse, Centers for Disease Control & Prevention, Atlanta, Georgia

S. Velsko, Lawrence Livermore National Laboratory,
  California: Nongenetic technologies.
T. Angel, Pacific Northwest National Laboratory, Richland,
  Washington: Proteomic complements to genomics.

R. Bull, Federal Bureau of Investigation, Frederick, Maryland:
  Potential host forensic signatures.

SESSION 6: Metagenomics
Chairperson: C. Fraser-Liggett, University of Maryland School of Medicine, Baltimore

G. Weinstock, Washington University School of Medicine, St.
  Louis, Missouri: Metagenomics for surveillance.
J. Ravel, Institute for Genomic Research, University of
  Maryland, Baltimore: Human microbiome.
Y. Fofanov, University of Houston, Texas: Software and
  analytical tools.

A. van Daal, Bond University, Gold Coast, Australia: Example
  of specific platform (Illumina HiSeq) capabilities to meet
  needs of microbial forensics.

SESSION 7: Bioinformatics
Chairperson: P. Keim, Northern Arizona University, Flagstaff

S. Velsko, Lawrence Livermore National Laboratory,
  California: What questions need to be answered?
J. Tiedje, Michigan State University, East Lansing: Population
  genetics issues.
R. Chakraborty, University of North Texas Health Science,
  Fort Worth: Statistical interpretation issues: Comparison to
  forensic human DNA.

T. Leighton, Children's Hospital, Oakland Research Institute,
  California: Next-generation DNA sequencing analysis of
  clonal variants, manipulated populations, and production
  process trace-back.
S. Velsko, Lawrence Livermore National Laboratory, California:
  Genetic inference on disease transmission networks.

SESSION 8: Data Sharing
Chairperson: D. Rock, College of Veterinary Medicine, Urbana, Illinois

T. Slezak, Lawrence Livermore National Laboratory,
  California: Forensic uses of microarrays or databases for
  sharing forensic data.
B. Budowle, University of North Texas Health Science Center,
  Fort Worth: Archives and database needs.

J. Smith, Penn State University, Pennsylvania; BioForensic
  Consulting, LLC, Edgewood, Maryland: Data exchange
  requirements and policies for sharing.
C. Cooke Jr., WINPAC, Washington, D.C.: Quality assurance
  for data generation.

SESSION 9: Summary
Chairperson: S. Schutzer, University of Medicine and Dentistry–New Jersey Medical School, Newark

B. Budowle, University of North Texas Health Science Center,
  Fort Worth and Traci Pals, U.S. Department of Homeland
  Security, Washington, D.C.: Prioritization of needs, recap,

wrap-up, strategies, and what we would like conveyed to the
community, stakeholders, policy makers, and summary for
manuscript.

Signaling through Ubiquitin

November 14–17

FUNDED BY   **The Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY   **R. Deshaies,** California Institute of Technology, Pasadena
                  V. Dixit, Genentech, South San Francisco, California
                  W. Tansey, Vanderbilt University Medical Center, Nashville, Tennessee

The object of this meeting was to analyze and discuss how protein ubiquitylation exerts its myriad biological effects within the cell. Although ubiquitin has traditionally been studied within the context of protein turnover, it is now clear that ubiquitylation refers to a complex set of posttranslational modifications that are interpreted by the cellular machinery to impact a broad range of biological processes, just one of which is proteolysis. Participants in the meeting discussed the spectrum of ubiquitin and related modifications, how they are recognized, and what they do. Discussions drew on experts in biochemistry, cell biology, computational biology, structural biology, genetics, and pathophysiology to identify common emerging themes in how ubiquitin and related proteins work.

Introductory and Welcoming Remarks: **J. Witkowski,** Banbury Center, Cold Spring Harbor Laboratory, New York
Introductory Remarks: **W. Tansey,** Vanderbilt University Medical Center, Nashville, Tennessee

SESSION 1: Signaling via Ubiquitin Chain Diversity and Recognition
Chairperson: T. Hunter, The Salk Institute, La Jolla, California

R. Cohen, Colorado State University, Fort Collins: Recognition of polyubiquitin chains.

K. Hofmann, Miltenyi Biotec GmbH, Gladbach, Germany: UBL receptors: At the crossroads of UPS and autophagy.

S. Polo, Institute of Molecular Oncology Foundation, Milan, Italy: Specificity in chain formation and recognition.

Z. Chen, University of Texas Southwestern Medical Center, Dallas: Ubiquitin signaling in the RIG-I antiviral pathway.

I. Dikic, Johann Wolfgang Goethe University Medical School, Frankfurt, Germany: Ubiquitin signaling networks.

K. Iwai, Osaka University, Japan: Linear polyubiquitination: A new regulator of NF-κB signaling.

J. Peng, Emory University School of Medicine, Atlanta, Georgia: Exploring ubiquitin pathways by quantitative proteomics.

SESSION 2: Signaling via Ub-Like Proteins
Chairperson: M. Hochstrasser, Yale University, New Haven, Connecticut

R. Hay, Sir James Black Centre, University of Dundee, United Kingdom: SUMO targeted ubiquitin ligases.
J. Huibregtse, University of Texas, Austin: The mechanism and function of ISG15 conjugation.
T. Hunter, The Salk Institute, La Jolla, California: Ubiquitin-SUMO cross-talk.
C. Lima, Memorial Sloan-Kettering Cancer Institute, New York, New York: Structure and mechanism in the SUMO conjugation pathway.

Y. Ohsumi, Frontier Research Center, Tokyo Institute of Technology, Yokohama, Japan: Two ubiquitin-like conjugation reactions essential for autophagy.
A. Huang, Sanofi-Aventis, Cambridge, Massachusetts: Regulation of axin stability through poly-ADP ribosylation: Linking Wnt pathway signaling activity, ubiquitination, and poly-ADP-ribosylation.

SESSION 3: Signaling to the Proteasome
Chairperson: J. Huibregtse, University of Texas, Austin

D. Finley, Harvard Medical School, Boston, Massachusetts: Editing of ubiquitin at the proteasome.
M. Glickman, Technion-Israel Institute of Technology, Haifa, Israel: Coordination of ubiquitin-processing factors at the proteasome.
M. Hochstrasser, Yale University, New Haven, Connecticut: Proteases of the ubiquitin system.

R. Kopito, Stanford University, California: Why do ubiquitin chains accumulate in neurodegenerative disease?
S. Murata, The University of Tokyo, Japan: Proteasome diversity.
R. Deshaies, California Institute of Technology, Pasadena: Nedd8 links active Cullin-RING ligases to p97 substrate processing machinery.

SESSION 4: Ubiquitin–Proteasome Signaling in the Nucleus
Chairperson: R. Hay, Sir James Centre, University of Dundee, United Kingdom

G. Rosenfeld, University of California, San Diego, La Jolla: Ubiquitylation, SUMOylation, methylation, and strategies in regulated transcriptional programs.
W. Tansey, Vanderbilt University Medical Center, Nashville, Tennessee: Ubiquitin and transcription.

H. Ulrich, Cancer Research UK, London Research Institute, Herts, United Kingdom: Ubiquitin and replication stress.

SESSION 5: Ubiquitin Signaling in Disease States
Chairperson: R. Kopito, Stanford University, California

A. D'Andrea, Dana-Farber Cancer Institute, Boston, Massachusetts: Regulation of the Fanconi anemia pathway to deubiquitination.

R. Youle, National Institute of Health, Bethesda, Maryland: Mechanisms of Parkin-mediated mitophagy.

SESSION 6: Ub-Ligases, Isopeptidases, and Their Substrates
Chairperson: C. Lima, Memorial Sloan-Kettering Cancer Center, New York, New York

V. Dixit, Genentech, South San Francisco, California: Ubiquitin modification in cancer.
W. Harper, Harvard Medical School, Boston, Massachusetts: Signaling through the ubiquitin–proteasome pathway.
M. Pagano, New York University School of Medicine, New York, New York: SCF ubiquitin ligases and cell proliferation.
P. Kaiser, University of California, Irvine: Interpretation of ubiquitin signal.

P. Matthias, ETH Zurich, Institute of Biochemistry, Switzerland: Regulation and substrates of Cullin-based E-3 ligases.
M. Rape, University of California, Berkeley: Role of lysine 11-linked ubiquitin chains in cell cycle control.
B. Schulman, St. Jude Children's Research Hospital, Memphis, Tennessee: Structural studies of Cullin-RING ligases.

DNA, Genetics, and the History of Mankind

The impact of molecular genetic studies has spread beyond the immediate discipline of biology, and as the study of genetic variation is essentially an historical study, DNA analysis has been applied to historical problems in related disciplines. For example, conclusions on the movements of early human populations based on linguistic and archeological data have been reinforced or modified by DNA analysis. Domestication of animals and plants marked one of the great transitions in human history, and genetic analysis is revealing how and where domestication took place. Genetic analysis may provide reliable information on personal relationships and identification that has been the subject of speculation, for example, Thomas Jefferson's family and identifying the bodies of the Tsar's family. Participants in this workshop discussed how genetic and DNA analysis can further our understanding of the history of human beings, and how to promote such studies.

Welcoming Remarks: J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

SESSION 1
Chairperson: M. Thomas, University College London, United Kingdom

K. Dobney, University of Aberdeen, United Kingdom and J.-D. Vigne, Muséum National d'Histoire Naturelle, Paris, France: Mammals to microbes: Role of genetics in exploring past bio-cultural dynamics using the fossil vertebrae record.

E. Willerslev, Natural History Museum of Denmark, Copenhagen: Early peopling of the New World and extinction of the megafauna.

M. Richards, University of Leeds, United Kingdom: Archaeogenetics and modern human dispersals.

C. Bustamante, Stanford School of Medicine, California: Sequencing admixed genomes and what the thousand genomes data are telling us.

A. Chakravarti, Johns Hopkins University School of Medicine, Baltimore, Maryland: Admixture: What it means for studying human populations.

B. Shapiro, Pennsylvania State University, University Park: Appropriately incorporating spatial and temporal information into genealogical analysis.

W. McCombie, Cold Spring Harbor Laboratory, New York: Human genetics in the era of "next-generation" sequencing.

SESSION 2
Chairperson: J. Buikstra, Center for Bioarchaeological Research, Arizona State University, Tempe

S. Pääbo, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany: The contributions of DNA studies: What we would not, and could not, have gained from other sources.

D. Reich, Harvard Medical School, Boston, Massachusetts: Evidence for gene flow from neanderthals into modern humans.

J. Hawks, University of Wisconsin, Madison: Natural selection, population growth, and human migrations in the early holocene.

M. Crawford, University of Kansas, Lawrence: The sequelae of Russian contact in the Aleutian archipelago: Molecular perspectives.

M. Zeder, National Museum of Natural History, Washington, D.C.: Documenting domestication: The intersection of archaeology and genetics.

A. Stone, Arizona State University, Tempe: The origins and spread of human tuberculosis.

SESSION 3
Chairperson: L. Madrigal, University of South Florida, Tampa

N. Wade, *The New York Times*, New York, New York: The evolution of human races and economic systems.

D. O'Rourke, University of Utah, Salt Lake City:

Consultation and consent: Cultural concerns in human population genetic research.

SESSION 4: Future Developments

J.-D. Vigne, Muséum National d'Histoire Naturelle, Paris, France: The Archaeozoology and Genetics (A&G) Working Group of International Council for Archaeozoology (ICAZ).

D. O'Rourke, University of Utah, Salt Lake City: The American Association of Physical Anthropologists.

J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory, New York

Discussion Points
- What might be done to foster cross-disciplinary interactions in these fields?
- What topics should be covered in a Banbury Center meeting in 2011?
- Any other points for discussion?

D. Micklos, S. Pääbo, D. Reich, B. Shapiro

DNA LEARNING CENTER

DNA LEARNING CENTER
EXECUTIVE DIRECTOR'S REPORT

Preparing students and families to thrive in the gene age

ADMINISTRATION	INSTRUCTION	BIOMEDIA
Lauren Correri	Jennifer Aiello	Anthony Biondo
Mary Lamont	Elna Carrasco	Cornel Ghiban
Valerie Meszaros	Jennifer Galasso	Uwe Hilgert
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	Ileana Rios	Todd Rocheford
	Tedi Setton	Jason Williams
	Jermel Watkins	Chun-hua Yang

Just as a unique pattern of bars in a universal product code (UPC) identifies each item for sale in a store, a DNA barcode is a DNA sequence that uniquely identifies each living species. This simple premise was galvanized into action during meetings held in 2003 at Cold Spring Harbor Laboratory's (CSHL) Banbury Center and funded by the Alfred P. Sloan Foundation. At these meetings, scientists agreed to use short, standard DNA sequences to classify all living species and launched the *International Barcode of Life Project* (iBOL).

Operating under the iBOL banner, collaborators from more than 150 countries are involved in "campaigns" to census diversity in different plant, fungal, and animal groups, including ants, bees, butterflies, fish, birds, mammals, fungi, and flowering plants, and within ecosystems, including the seas, poles, rainforests, and coral reefs. The 10-year Census of Marine Life announced in October the first comprehensive list of more than 190,000 marine species, involving 2700 scientists and identifying 6000 potentially new species.

DNA barcoding is also important in detecting food fraud and products taken from conserved species. In advance of formal DNA barcoding, in 1996, Rob DeSalle of the American Museum of Natural History (AMNH) used related methods to find that five of 23 samples of caviar purchased in New York City (NYC) were mislabeled and included three samples from threatened sturgeon species. The Congressional Research Service recognizes that more than a dozen commercial fish are often mislabeled, passing off cheaper fish for expensive varieties. In 2008, Trinity High School students worked with Mark Stoeckle of The Rockefeller University and George Amato of the AMNH to show the pervasiveness of fish fraud in NYC. They found that 25% of 60 seafood items purchased in grocery stores and restaurants were mislabeled as more expensive species. One mislabeled fish was an endangered species, the Acadian redfish.

Urban Barcode Project

DNA barcoding came full circle to New York, when in December we received a $300,000 grant from the Sloan Foundation for the *Urban Barcode Project*, a science competition spanning the five boroughs of NYC. Student research teams will use DNA barcoding to explore biodiversity in the city environment, including:

- Sampling biodiversity in a park, garden, office, or school.

- Checking for invasive plant or animal species.

Logo for the *Urban Barcode Project*

- Monitoring animal movements or migrations.
- Identifying exotic or endangered food products in markets.
- Detecting food or product fraud.

The competition is open to NYC high school students enrolled in grades 9–12. Teams of two to four students will work with a teacher sponsor to submit a project proposal for a June or October 2011 deadline. Proposals will be judged for originality, creativity, relevance, plausibility, and scientific merit. The top 100 teams will be invited to compete in the *Urban Barcode Project*. Teams must complete their projects by the spring of 2012 and will present their work at a project symposium. The best overall project will win the Grand Prize of $10,000, and an additional $10,000 in runner-up prizes will be awarded.

Sponsoring teachers must participate in a 6-hour training session, which will dovetail with our existing training program sponsored by the Howard Hughes Medical Institute (HHMI). Each successful team will have free access to everything needed for their DNA barcode experiments, including equipment, protocols, and reagents. Five equipment footlockers will be available for use by individual schools or for groups of nearby schools to share. During the term of the project, we expect to process ~10,000 DNA sequences. Teams may work on their projects at summer workshops and Open Lab days at designated locations.

Teams will be assigned a mentor from a NYC university, museum, or other scientific institution to answer technical questions and provide advice. Many mentors will be drawn from five NYC institutions that are collaborating on the project: AMNH, New York Academy of Sciences, New York Botanical Garden, Prospect Park Zoo, and The Rockefeller University. The project's scientific advisor, Mark Stoeckle, is a member of The Rockefeller Program for the Human Environment. In addition to demonstrating the educational promise of DNA barcoding, Dr. Stoeckle was one of the organizers of the 2003 Banbury meeting that launched the field of DNA barcoding and serves on iBOL's Scientific Steering Committee.

A dedicated Internet microsite (www.urbanbarcodeproject.org) supports all phases of the project. This "online lab notebook" includes a video introduction to the barcode experiment, downloadable PDF lab protocols, teacher preparation, bioinformatics tools, and multimedia resources. Video interviews with scientists, animations, and video podcasts (vodcasts) describe the science and applications of DNA barcoding, and an active news feed and a link to Dr. Stoeckle's *Barcode Blog* provide up-to-date perspectives on DNA barcoding. Student DNA sequences are automatically uploaded to the "Blue Line" of *DNA Subway*, an online tool for DNA barcode and phylogenetic analysis developed as part of the National Science Foundation (NSF)-funded *iPlant Collaborative*. A Google Maps utility will track biodiversity discovered by student experiments, and students will highlight their projects through the social networking website, *Facebook*.

Student DNA Sequencing

A decade ago, the DNALC developed a similar program—with simplified biochemistry and a bioinformatics work flow—to allow students to analyze a portion of their own mitochondrial (mt) chromosome. Using a kit distributed by Carolina Biological Supply Company, students isolate DNA from cheek cells and then use polymerase chain reaction (PCR) to amplify the mitochondrial control region. Student samples are sent by overnight mail to the DNALC, where student interns prepare them for sequencing. The processed samples are then sent to the CSHL Sequencing Facility in Woodbury, and finished DNA sequences are uploaded to the *Sequence Server* database at our *BioServers* website. There, students visualize their sequences and use software to align them with mitochondrial sequences from modern humans and extinct hominids to explore human genetic diversity and evolution.

The donation of sequencing reagents by Applied Biosystems of Foster City, California has made it possible to provide students this *Sequencing Service* free of charge. Since 1998, a total of 49,217 student DNA samples have been sequenced, and 1.3 million students and teachers have used the supporting *Bioservers* website. We continued the *Sequence Service* in 2010, processing 7128 student DNA samples received from 93 high schools and 57 colleges and universities.

The cost of commercial sequencing, however, has decreased so dramatically during the last year that in December, we ceased sequencing on site and shifted all sequencing to *Genewiz*, a global contract research organization with expertise in sequencing. We negotiated a much-reduced price of $3.00 per sample, which is less than our cost of sequencing with free reagents and student labor! This arrangement anticipates the increased volume of sequencing demanded by our *Urban Barcode Project*, as well as growing interest in using DNA sequencing in education. Student sequences are uploaded to our server within 48 hours of receipt by *Genewiz*, a dramatic improvement over the 2-week turnaround we were previously able to provide.

iPlant Collaborative

The *iPlant Collaborative* is a consortium headed by the University of Arizona and CSHL to develop a computer (cyber) infrastructure to support plant research. *iPlant* aims to develop tools and interfaces that will provide scientists with easy access to large-scale plant data sets and high-powered informatics tools. Working Groups composed of *iPlant* staff and plant community members are currently building "discovery environments" to help plant scientists solve two "grand challenges": (1) produce an *iPlant* Tree of Life (iPToL) encompassing ~500,000 plant species and (2) explore the genotype-to-phenotype continuum in plants (iPG2P).

Education, outreach, and training (EOT) is integral to *iPlant*'s mission of helping plant scientists make the best use of computer infrastructure to solve biological problems. During the first 2 years of the project, EOT worked in parallel with the Grand Challenge teams to create an educational Discovery Environment that allows students and teachers to work with the same data and use the same tools at the same time as high-level plant researchers. This culminated in the release in March of *DNA Subway*, a student-centered platform for gene annotation and comparison.

DNA Subway uses the metaphor of a subway map as an appealing interface to sophisticated informatics tools. "Riding" on either of the initial lines, students can predict and annotate genes in up to 150 kb of DNA (Red Line) or identify homologous, or related, sequences in whole genomes (Yellow Line). By year's end, a third "Blue Line" to analyze DNA barcodes and other short DNA sequences was mostly complete. This line offers the types of sequence analysis that precedes the massive tree construction envisioned by the iPToL

DNA Subway's Blue Line

Grand Challenge, simplifying sequence alignment and construction of phylogenetic trees. The Blue Line will support the *Urban Barcode Project* in NYC and a nationwide *iPlant* program to encourage students to identify plants using the barcode region of the *rbcl* gene found in chloroplasts. Thus, *DNA Subway* provides opportunities for students to discover basic principles of genome biology while embarking on independent research. By December 2010, the site had 742 registered users and had received 14,535 unique visits.

Acting on recommendations of an NSF site visit in 2010, EOT began to expand its role and became a Working Group analogous to those within the iPToL and iPG2P Grand Challenges. The EOT Working Group (EOTWG) includes core *iPlant* staff, collaborators, and community members from each of the Grand Challenge areas. The EOT Working Group also aims to increase the number of scientists who use and contribute to *iPlant* Discovery Environments and tools. Toward this goal, we are extending our successful workshop program to high-level researchers and are encouraging participants to bring their own data to analyze at workshops. This collaborative effort will help us to develop case studies of interesting research questions that can be addressed with *iPlant* Discovery Environments.

As part of this expanded role, EOT multimedia staff at the DNALC took on the task of redesigning the *iPlant* website to increase its appeal and utility (www.iplantcollaborative.org). This included the development of podcasts that introduce *iPlant* and the significance of the two Grand Challenges. The podcasts are available at the *iPlant* and DNALC websites and on *YouTube*. In the coming year, we will develop intuitive interfaces to data sets and simplified work flows that make *iPlant* cyberinfrastructure available to a wide range of users. One project will repackage the iPG2P work flow for high-throughput sequencing as a "Green Line" on *DNA Subway*, allowing analysis of RNA data, including transcriptome and RNA-Seq data. We will also develop an *Orphan Data* interface that will match underworked data sets with students and faculty who would like to contribute to original research.

iPlant Collaborative redesigned homepage

Harlem DNA Lab

Genetics and biotechnology occupy major parts of required science courses and exams taken by virtually all NYC students in the eighth through tenth grades. However, teachers have received little training and few resources to deliver hands-on labs that could make these topics come alive for students. Thus, the objective of our HHMI program is to use our expertise to prepare NYC teachers to teach a set of six labs that target key genetics and biotechnology concepts: *DNA Structure and Isolation*, *Variability and Inheritance*, *Bacterial Transformation*, *Protein Isolation*, *DNA Analysis and Forensics*, and *Analysis of Human DNA Variations by PCR*.

The lab program is supported by an Internet microsite, *Lab Center*, which is a virtual classroom into which a DNALC staff member enters to make a video introduction for each lab. By clicking on various objects on the whiteboard and desk, students can access (1) interactive and PDF versions of the experiment, (2) follow-up activities, (3) scientist interviews, (4) animations, and (5) selected links. In 2010, we began development of the final element of *Lab Center:* a series of vodcasts to highlight how each of the target labs relates to research done at New York–area institutions. Each *New York Story* will be largely produced by a team of NYC students. The initial vodcast, on green fluorescent protein (GFP) and bacterial transformation, was developed by a six-student team from Brooklyn International School and organized by HHMI Teacher Fellow and Assistant Principal

Kathleen Rucker and science teacher David Conneely. In parallel with a DNALC videographer, students used flip cameras to record in their school and at locations at Columbia University, where they interviewed Nobel laureate Martin Chalfie.

Student at *Harlem DNA Lab*

During the year, we also implemented an important new element of in-school support for NYC teachers. A grant from the Lounsbery Foundation supported the purchase of equipment to outfit 15 biotechnology footlockers that contain all of the equipment and expendable supplies needed to teach each target lab. The kits are available to any teacher who has undergone HHMI training. At $50 per class of 32 students, each footlocker is less than half the cost of a commercial kit. In their inaugural year, the kits extended lab experiences to 1455 students, about half the number accommodated in field trips to the *Harlem DNA Lab*. To accelerate footlocker use, we waived the restocking fee for most schools and provided free footlocker shipping. To further support teachers as they implement the labs, we recruited a part-time instructor at year's end to accompany footlockers to schools and help set up the equipment or coteach the lab activities.

We also tore a page out of the DNALC's own play book when we created a *Charter Membership Program* for *Harlem DNA Lab*. The DNALC was started in 1985 with multiyear memberships from local Long Island school districts. Adopting this model in the spring, we welcomed Trinity School and The Chapin School as Charter Members of our Manhattan operation. Each school receives an exclusive set of benefits to develop a sequenced program of accelerated science opportunities for students. The program launched in June, with in-school *Fun with DNA*, *DNA Science*, and *Human Genomics* workshops taught by DNALC instructors. The collaboration continued through the academic year with focused faculty development, field trips to the DNALC, and assistance with student research.

Although *Harlem DNA Lab* prospered in the face of the recession, we became increasingly concerned with the continued devolvement of the NYC Department of Education (DOE). In the spring,

Student at *Harlem DNA Lab*

the DOE quietly disbanded its entire science supervisory department, with whom we and other organizations collaborated to enrich science programs. It has also reassigned key science discretionary funds to English and math, for which student test scores are closely tracked for federal aid. The administration has pushed responsibility for science advancement onto loosely allied networks of schools, expecting them to self-organize without access to funds. This explains why it is difficult for teachers to find even the modest funds needed to take a field trip to the *Harlem DNA Lab* or to restock reagents for a footlocker kit that they can borrow from us.

Our host school, the John S. Roberts Educational Complex, is emblematic of the instability faced by many schools in NYC. It has been adversely affected by administrative shuffling, with two schools phased out and two schools phased in during our tenancy. Simple tasks, such as securing resources and teacher cooperation, have become more difficult. Student discipline and security have become major concerns as enrollment increases in each of the tenant schools. These situations impact the field trip experience for our visitors.

Reaching Underrepresented Minorities

It is a sad fact that Hispanic and African Americans perform poorly in science and are underrepresented in scientific fields when compared with Caucasians. Hispanic and African Americans perform

~20% lower in science than Caucasians on the International Assessment of Education Progress, a respected benchmark for student achievement. This disparity holds true in grades 4, 8, and 12. According to the National Science Board, these groups are underrepresented in postsecondary science education and science careers compared to their proportion of the overall U.S. population (24%). Underrepresented minorities received 17% of bachelor's degrees, 13% of master's degrees, and 7% of doctorates in science and engineering fields in 2007. They accounted for only 10% of college-educated persons employed in science and engineering occupations and received salaries that averaged 25% less than those of Caucasian workers.

The National Science Education Standards and other studies emphasize that students need to be engaged in the process of science, asking questions, forming hypotheses, designing experiments, collecting data, analyzing results, and forming conclusions. Underrepresented minorities—who are concentrated in low-achieving schools and who may receive less science "boosting" at home—have less access to hands-on experiences that help them to learn about science in the same way as scientists.

The DNALC takes seriously the challenge of increasing minority representation in science. The *Urban Barcode Project* and our *Harlem DNA Lab* were established for the express purpose of providing minority and disadvantaged students—and their teachers—the same opportunities for laboratory-based learning as those offered students in wealthy suburban school districts. Statistics for 2010 show that our Manhattan operation is reaching this target audience. Hispanic and African Americans made up 69% of students visiting the *Harlem DNA Lab* and 41% of teachers trained under our HHMI program. The strong Harlem numbers, combined with a policy of conducting many teacher-training workshops at institutions serving minorities, maintained underrepresented minorities at 25% of 696 teachers trained at 1–10-day workshops.

Doing the best job in science education for underrepresented minorities also requires that an institution practice what it preaches—and provide role models for students to emulate. Thus, during the last several years, the DNALC has hired four exemplary African American and Latino educators, who now compose 33% of the instructional staff. Jermel Watkins, Ph.D., began his science career as a DNALC intern and completed his graduate training at Stony Brook University. Jason Williams moved into education after research stints in two CSHL laboratories. Ileana Rios, Ph.D., attended elementary school several blocks away from the *Harlem DNA Lab* and completed her graduate training at City University of New York. Our newest staff member, Kerri-Ann Matthews, brings experience as an informal science educator with the New York Hall of Science and other science exhibitions.

Internet Strategies and Visitation

The September issue of *Wired* magazine ran the full-page headline, "The Web is Dead." The lead article made the point that the World Wide Web (WWW)—navigated by browsers such as Explorer, Firefox, Safari, and Chrome—now accounts for less than 25% of total Internet traffic. The vast majority of Internet bandwidth is consumed by other types of communication, notably, e-mail, virtual private networks (VPNs), voice-over Internet, music and video sharing, and active gaming. The article also referred, metaphorically, to the struggle between the chaotic, "everything-all-the-time (from-everyone)" world of websites—dominated by search engines such as Google—and the "what-you-need-when-you need-it (from-someone-you-trust)" world of cell phone applications (apps) and social media—dominated by sharing software such as Facebook and Apple iTunes. The DNALC now finds itself in this struggle to adapt to the changing Internet landscape.

The DNALC benefited from its early entry into the Internet world. At a time when there were only 10–25 million active websites on the Internet, websites we developed in the late 1990s and early 2000s rapidly built audiences. Overall visitation increased steadily, peaking at 7.1 million in 2007. However, our visitation declined and then leveled at ~6 million in 2008–2009. In less than 5 years,

(*Left*) The refreshed *DNA from the Beginning* website; (*right*) DNALC *YouTube* channel

the web had grown to more than 100 million active sites; the DNALC and other small-content providers now found it increasingly difficult to build and maintain audiences.

In the face of an exponentially crowded web, search engines such as Google became the primary arbiters of website visitation. We therefore embarked on an ambitious program to redesign our older websites to make them more "visible" to search engines, a process called search engine optimization (SEO). DNALC.org, the home site from which all other DNALC websites are reached, was the first to be revamped according to SEO principals. Relaunched in September 2009, it saw a 24% increase in visitation in 2010. *DNA from the Beginning*, our first major website, is now undergoing an even more extensive SEO makeover, and we anticipate a similar bump in visitation when it is relaunched in spring 2011.

In parallel with SEO, we have turned to other channels—applications for handheld devices ("apps") and social media—to broaden the audience for our multimedia products. Our *3D Brain*— a detailed rendering of the human brain that can be rotated and explored in three dimensions—provides a remarkable example of the way in which rechanneling content can increase viewership. Originally developed as part of the website *Genes to Cognition (G2C) Online*, which debuted in spring 2009, *3D Brain* was later launched as a stand-alone iPhone, iPod, and iPad application. It rose to number seven of 7,100 education apps and number one among 250 iPad apps. In 2010, the web version of *3D Brain* received 54,868 visits, and the app version had 413,874 downloads! Feedback on the *iTunes* site shows, as one would expect, strong use by college students and faculty. However, it is also used by medical professionals: "Excellent! I use this with clients to help them see where some of their PTSD (posttraumatic stress disorder) symptoms are coming from. Has been very helpful for them."

During the year, we continued an aggressive program to develop a DNALC channel on *YouTube*, the site where most people find and share videos. Visitation surged following the addition of nearly 100 new videos, including a collection of three-dimensional molecular animations created for the *DNA Interactive* website by 2010 McArthur Prize–winner Drew Berry. Our collection of 184 videos received 280,503 views in 2010. Blogs, supporting each of our major content sites, generated an additional 283,843 web visits.

As a result of the SEO makeover for the DNALC homepage plus aggressive moves into apps, *YouTube*, and blogs, Internet visitation rose 14% to 7.1 million in 2010, equaling the 2007 peak. App downloads, *YouTube* views, and blog views contributed 933,220 visits, or 13% of total Internet traffic. The amount of data served by DNALC websites surged 35% in 2010, to 6669 gigabytes (GB), or 6.5 terabytes (TB). DNALC.org accounted for 43% of all data served.

Visitation was also augmented by a grant from Google AdWords, through which we receive free "sponsored" links on Google searches. The AdWords account contains a set of keywords for each DNALC website. When someone searches for one of the keywords, an ad for the related DNALC site is displayed and logged as an "impression"; a "click-through" is logged when the link is followed. (Each click results in a visit to one of our websites.) AdWords generated 20,185,997 impressions of DNALC ads, resulting in 133,034 website visits. This advertising is valued at $99,438. *G2C Online* is our most successful AdWords campaign, with two-thirds of 62,952 click-throughs resulting from searches for "brain."

Teacher Training

Since trekking from New York to California in our mobile *Vector Van* in the summer of 1986, the DNALC has maintained a unique ability to provide sophisticated lab and computer training at essentially any site around the world. The year 2010 was the zenith of off-site training, with 940 high school and college faculty participating in professional development activities conducted at 27 sites across the United States and Canada. (For a complete list of training activities and host sites, see the tables at the back of this report: "2010 Workshops, Meetings, and Collaborations.")

With funding from HHMI, and in collaboration with the NYC Department of Education, 290 teachers participated in 52 workshops conducted at the *Harlem DNA Lab*. Ease of scheduling and quick completion of P-Credits (graduate equivalent) and Professional Development hours (required to maintain a New York State teaching license) made the summer workshops more popular than those held during the academic year.

In our role as education lead of the *iPlant Collaborative*, we introduced *DNA Subway* to 187 college teachers at 10 2-day workshops hosted at academic institutions around the country. An additional 188 educators learned about *DNA Subway* at short courses and demonstrations at the meetings of the American Society of Plant Biologists, Botanical Society of America, DOE's Joint Genome Institute, *iPlant Collaborative*, National Association of Biology Teachers, and USDA Plant and Animal Genome research program.

With funding from NSF's Course, Curriculum, and Laboratory Improvement (CCLI) program, we continued our effort to bring compelling RNA interference (RNAi) experiments into college classrooms via an experiment- and bioinformatics-rich curriculum. In 2010, 54 faculty attended follow-up workshops designed to increase confidence with our RNAi curriculum and update participants on new developments in the field. The training program is supported by the *Silencing Genomes* website that includes all experiments and reagent recipes, and a free biological library, that includes all needed bacterial and *C. elegans* strains and more than 100 vectors developed by workshop participants to silence worm homologs of human genes. To date, more than 1500 strain orders have been filled and have been used with a reported 12,500 students. Two stand-alone kits derived from the program have been released by Carolina Biological Supply Company, with a third to be released early in 2011.

With support from the National Institutes of Health (NIH) Science Education Partnership Award (SEPA) and the Hewlett Foundation, we continued to disseminate two large-scale Internet sites at 1- to 2-day workshops. Under the SEPA program, 71 high school and college faculty attended five workshops to learn how to use the *Inside Cancer* website to enhance teaching of cancer cell biology in health, general biology, and advanced biology classes. In addition, 18 Regional Fellows taught 228 educators at 22 second-round workshops. Five workshops on our Hewlett-sponsored website, *G2C Online,* drew 88 high school and college educators.

Continuing our long-term relationship with the Singapore Ministry of Education, two Singaporean biology teachers participated in HHMI Leadership training in July. Then, in the fall, we hosted four primary school teachers (grades 4–6). During their 2-week attachment, they observed and cotaught student field trips and in-school programs. They were also immersed in the *Fun with*

DNA and *World of Enzymes* curricula during small workshop sessions with DNALC instructors. Each of the teachers developed a plan for sharing their new knowledge when they returned home.

Program Evaluation

In 2010, we initiated nationwide experimental studies to evaluate how two of our websites contribute to science instruction in the classroom. The *Inside Cancer* website explores the molecular basis of cancer. The evaluation study, funded by an NIH SEPA Phase II grant, involved four teachers who used the website to teach topics such as the cell cycle, mutations, and genetic disease to 199 students in Illinois, Missouri, and Maryland. The *G2C Online* website explores the molecular basis of brain function and dysfunction. In the evaluation, funded by the Dana and Hewlett Foundations, five teachers used *G2C Online* to teach nervous system function and psychiatric disorders to 146 students in Missouri, Minnesota, New York, and North Carolina. (An additional 12 teachers and more than 900 students will participate in both studies in 2011.)

Pre- and posttest surveys—used by students across all study sites—measure changes in basic genetics literacy and attitudes toward science. Content tests, developed locally by each teacher, measure specific knowledge gained. To control for differences between teachers and students, we used a repeated measures study design, where a participating faculty member teaches the same topic to two different classes—one class receives classroom instruction only (control) and the other receives classroom instruction plus supplementary use of the website (treatment). For a second topic, the classes switch roles as treatment and control. Students were also asked questions to determine whether they prefer to explore information as network-related items or follow a suggested linear "story."

Considerable effort has focused on evaluating *Silencing Genomes*. With a response rate of 75%, follow-up surveys show that the majority of 262 college faculty who received workshop training completed *Silencing Genome* labs in their classrooms, reaching 3926 students and sharing them with 156 other teachers. An in-school study is matching experimental classes that do RNAi laboratories with control classes that learn equivalent topics without labs. Pre- and posttests measure changes in student attitudes, genetic literacy, and detailed knowledge of RNAi. To date, six teachers and 76 students have participated, with an additional 10 teachers and more than 400 students expected in 2011. Preliminary analyses are encouraging, with experimental students showing significant gains in knowledge of RNAi (12.0%, p = .0003).

Student Programs

In 2010, we continued to be the world's largest provider of hands-on learning in genetics and molecular biology. Twenty thousand students performed experiments at the Dolan DNALC, DNALC *West*, and the *Harlem DNA Lab*, and 10,856 students received in-school instruction by DNALC staff. High school students composed 31% of lab visitors (9417). Most of the 21,442 middle school visitors participated in our *Genetics as a Model For Whole Learning* Program. A grant from the William Townsend Porter Foundation provided scholarships for 1057 Hispanic and African America students to attend labs at *Harlem DNA Lab*. Grants from Bank of America, TD Bank, and National Grid provided lab visits and in-school instruction for 2349 students from underserved schools in Queens and on Long Island.

Summer camps drew 908 students to the Dolan DNALC, DNALC *West*, and *Harlem DNA Lab,* and to The Trinity and Chapin Schools, with two new workshops proving to be popular. DNALC staff member Jen Aiello drew on her recent undergraduate degree in forensic science to develop *Forensic Detectives*. This intermediate-level course for eighth and ninth graders includes labs on fingerprinting, forensic anthropology, toxicology, ballistics, and criminalistics. *Silencing Genomes* is a spin-off of the NSF teacher-training program of the same name, initiated by DNALC instructor

Participants in a *World of Enzymes* summer camp

Bruce Nash. This course introduces the Nobel Prize–winning technology of RNAi in the model organism *Caenorhabditis elegans.*

Great Moments in DNA Science Honors Seminars, conducted in the spring, drew 211 top high school students from Long Island for three in-depth presentations of current biological research. Dr. Chih-Shan Jason Chen of Memorial Sloan-Kettering Cancer Center presented his research on the genetics of *Dermatofibrosarcoma protuberans* (DFSP), an aggressive soft tissue tumor that often relapses after treatment. Dr. Damon Love of Weill Cornell College of Medicine discussed his work with a protein called Chibby, which is required for proper lung development in the fetus. Dr. Zuzana Zachar of Stony Brook University discussed recent discoveries in cancer cell metabolism and introduced "thioctans," a new class of chemotherapeutic agents that target cancer cells while sparing healthy ones.

The *Harlem DNA Lab* participated in several science education events around NYC. Children and parents extracted DNA from bananas at our booth at the World Science Festival in Washington Square Park and at the Morningside Area Alliance 2010 STEM Expo in Harlem. With support from the Porter Foundation, high school students from the *Mentoring in Medicine* program attended a week-long *DNA Science* camp. Founded by Dr. Lynne Holden, the program seeks to improve diversity in medicine by providing academic opportunities to underrepresented minority students.

Saturday DNA! drew 203 participants to monthly events held during the fall, winter, and spring. Two-hour sessions were offered at two age levels: one for children ages 10–13 with an accompanying adult and one for ages 14 and above. Topics presented by the DNALC's education and scientific staff covered such diverse topics as DNA barcoding and the genetic basis of smell.

Partnerships and Graduate Training

In the spring, we graduated our fourth class of *Genome Science*, our partnership with Cold Spring Harbor High School (CSHHS). Coinstructed by DNALC staff and CSHHS biology teacher Martin Glynn, this college-level course brings students to the DNALC for their final two class periods each day. As in previous years, the course emphasized critical thinking and included experiments and independent projects across a range of biological systems. One unit looked for evidence of genetic modification in common foods and annotated newly sequenced genes in rice. Another unit used students' own DNA to explore the molecular basis of simple traits and trace human origins. The final unit used RNAi to turn off genes in the roundworm *C. elegans* and study worm equivalents of genes involved in human diseases.

In the fall, the fifth year of *Genome Science* began under the tutelage of DNALC staff along with a new CSHHS staff member, Jaak Raudsepp. This was a comfortable change because Jaak is a graduate of our former *Leadership Institute*, the rigorous 3-week program for the nation's best biology teachers. Always a proving ground for our latest experiments, the CSHHS students were the first to use DNA barcoding to identify plants collected in the local area. In preparing essays on modern parallels to the eugenics movement of the early 20th century, students also visited the CSHL Research Archives to examine historical documents remaining from the former Eugenics Record Office.

As part of a reinvigorated collaboration with the Center for Science and Mathematics Education (CESAME) at Stony Brook University, CSHHS students completed a module on protein modeling. Using methods developed by Tim Herman at the Milwaukee School of Engineering, students developed three-dimensional molecular models to illuminate how chemotherapeutic drugs interact with protein receptors on the surface of cancer cells. Using data from primary research papers, students identified important parts of the drugs and receptors. They then used three-dimensional modeling software to highlight key atoms involved in molecular interactions. The modified structure files were sent to CESAME, where detailed atomic resolution models of the proteins were manufactured on state-of-the-art prototyping printers. (These machines are essentially laser printers that layer droplets of polymer resin in three dimensions.) Students will present their models and explanatory posters at the spring 2011 CSHL meeting, "The Biology of Cancer."

During their second semester, graduate students at the CSHL Watson School of Biological Sciences work under the guidance of experienced DNALC instructors. The graduate students work in pairs during 12 half-day sessions, learning from and leaning on one another as they develop effective teaching techniques. During the first phase of training, students observe a DNALC staff member teaching a laboratory class and then organize a lesson plan that integrates their own perspectives. During the second phase, students join the DNALC staff member to coteach parts of a laboratory class. After critiques of their lesson plans and coteaching experience, the students move on to independently teach an entire lesson. After completing rotations with middle- and high-school-level experiments, students deliver three additional lessons to demonstrate mastery of teaching and class management skills.

In October, we began a collaboration with the New York Academy of Sciences (NYAS), one of the oldest scientific institutions in the United States and a leading organizer of scientific meetings. The NYAS developed a new science mentoring program in which graduate students and postdoctoral researchers from NYC research institutions present science activities to students in grades K–12. DNALC instructors trained 22 mentors in effective strategies to deliver hands-on genetics activities in after-school settings.

Staff and Interns

We were sad when Dr. John Connolly left in February to evaluate clinical trials at the Center for Applied Genomics, Children's Hospital of Philadelphia. After receiving a Ph.D. in neuropsychology at Trinity College, Dublin, John came to the DNALC in 2005 to develop our innovative website *G2C Online*, for which he interviewed more than 80 scientists. As Multimedia Coordinator, he became indispensable in managing many aspects of our Internet venture—developing the Landeau Multimedia Studio and launching regular blogs for each of our major content sites. Notably, he moved us into the world of apps with his spectacularly successful *3D Brain*. We will miss his Irish brogue, which often lightened the office atmosphere.

The multimedia staff returned to full force in November, with the arrival from "down under" of Multimedia and Evaluation Manager Dr. Amy Nisselle. After attending the DNALC's presentation at the 19th International Congress on Human Genetics in Melbourne in 2003, Amy decided to pursue her Ph.D. in multimedia genetics education. In 2008, she spent 3 months at the DNALC, evaluating the *G2C Online* website as part of her thesis research. In addition to managing multimedia

Mohammed Khalfan and
Amy Nisselle.

projects, Amy will also increase our academic profile in the world of science and technology education.

Our growing involvement in the *iPlant Collaborative* led to the recruitment in November of Computer Programmer Mohammed Khalfan. After receiving a B.S. degree from the University of Toronto, he received a Master's degree in bioinformatics and computational biology at the University of Newcastle upon Tyne, United Kingdom. His initial projects include building the "Blue Line" (phylogenetics) of *DNA Subway* and redeveloping the *iPlant* website in Drupal, an open source content management system (CMS).

Our multimedia group also benefited from fresh ideas from young people. In the fall, we welcomed Todd Rocheford as Design Assistant and Videographer. Todd has an undergraduate degree in video production from the University of Canberra, Australia. We met Todd through his father, Torbert, a Purdue geneticist who developed vitamin-A-fortified corn. After filming a documentary on his father's work in Africa, Todd realized that he wanted to develop educational videos, leading to his position at the DNALC. Undergraduate intern Tony Biondo was promoted to Junior Programmer, based largely on the initiative that he took in developing an Android version of our *3D Brain* app. Tony is a sophomore majoring in computer science at Stony Brook University. An active supporter of open-source software, he won the Student Cluster Competition of the 2009 International Conference on High-Performance Computing at Portland, Oregon. Chris Weidler (Farmingdale State College) continues to support the *BioMedia* Group and was especially productive on the *DNA from the Beginning* upgrade.

Our internship program continued to draw some of Long Island's most talented high school and college students, engaging them in science research and providing practical laboratory experience. We were pleased to accept several new high school interns this year: Devika Gupta (Farmingdale), Jueng Woen Kim (Hauppauge), Yasin Muhammad (Lawrence Woodmere Academy), H. Alan Phipps (Portledge School), David Streitman (Syosset), and Young Joon Suh (John Glenn). The new hires joined a dedicated group of returning interns: Laura Bergsten (Cold Spring Harbor), George Economou (Syosset), Jack Greenfield (Oyster Bay), Lindsay Hochberg (Oyster Bay), Max Vaysman (Commack), and Pamela Wax (Harborfields). We bid farewell to a number of high school interns as they left for their freshman year at college: Anouva Kalra-Lall (Case Western Reserve University), Emily Troge (Brandeis University), Sara Wienclaw (University of Delaware), and Kevin Wu (New York University).

In addition to regular prep duties, college interns help to fulfill requests from faculty nationwide for student DNA sequencing and RNAi targeting vectors. Three former high school interns returned to assist with summer workshops: Kaitlin Watrud (Gettysburg College), Arielle Scardino (City College of New York), and Seth Shortz (Emory University). New college interns starting in 2010 were David Dopfel (Stony Brook), Sarah Justvig (Georgetown), Sulaiman Usman (New York Institute of Technology), Lina-Mari Varghese (Stony Brook), and Katherine Villalon (John Jay). Annie Laurie Benzie (Adelphi University) left the DNALC for an internship at Bellevue Hospital.

Many of our high school interns are involved in science research and compete in state and nationwide science competitions. Anouva Kalra-Lall was an Intel semifinalist and a top-four finalist for the Neuroscience Research Prize. Young Joon Suh took first place in chemistry at the New York State Science and Engineering Fair. Laura Bergsten was accepted into the CSHL Partners for the Future Program.

Additionally, DNALC staff served as mentors to local high school students working on independent projects. Elliot Horlick (Cold Spring Harbor) worked on two projects with Dr. Jermel Watkins: sunblock (SPF) protection against UV-induced DNA damage in yeast and the protein complement (proteome) of squamous cell carcinoma. Daniel Krumholz (Oyster Bay) worked with

Dr. Bruce Nash to use RNAi to "knock down" genes in *C. elegans* that are homologous to human genes involved in serotonin signaling.

Expert Advisors and Corporate Support

We are fortunate to have high-level support from two advisory bodies: the DNALC Committee and the Corporate Advisory Board (CAB). The DNALC Committee consists of community leaders and members of CSHL senior management and the Board of Trustees, who oversee strategic development including capital funding and the evolution of satellite locations in North America and beyond. The CAB provides liaison to the Long Island and NYC business communities; its annual fund campaign and golf tournament contributed $155,000 in 2010.

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Dan Decker, Eppendorf North America
Robert Dickstein, Ph.D., Pall Corporation
David Epstein, Ph.D., OSI Pharmaceuticals, Inc.
Candido E. Fuentes-Felix, M.D., F.A.C.S.
David Garbus, Huntington Business Products Centre
Lawrence Goodman, Curtis, Mallet-Prevost, Colt &
  Mosle LLP
Mary Jane Helenek, Luitpold Pharmaceuticals
Arthur D. Herman, Herman Development Corporation

Diana Hoadley, JP Morgan
Robert Isaksen, Bank of America
Neil M. Kaufman, Davidoff, Malito & Hutcher
John C. Kean III, Kean Development Corporation
Norman Kelker, Enzo Life Sciences
Robert Keller, National Grid Foundation
James E. Mattutat, Ingleside Investors, Inc.
John Molloy, H2M
John Passarelli, M.D.
Patricia Petersen, Daniel Gale Sothebys Real Estate
Frank Posillico, Alliance Real Estate Corp
Christiaan B. Reynolds
William D. Roche
Raju Sarwal, M.D.
Charles R. Schueler, Cablevision
Lee Shuett, Nikon Instruments
Kurt Timmel, Marsh USA
Jeffrey K. Tupper, U.S. Trust Company of New York
Robert Van Nostrand
Hans Zobel, Ziehm Medical LLC

426 DNA Learning Center

2010 Workshops, Meetings, and Collaborations

January 5	Site visit by William Mak, Hong Kong Biotechnology Education Resource Center and Mobile Lab, Hong Kong University, Pokfulam
January 12	Site visit by Denise Philpotts, Trinity School, *Harlem DNA Lab*
January 14	NSF *Plant Collaborative*, Genotype 2 Phenotype Steering Committee Meeting, San Diego, California
	HHMI Professional Development Workshop, "PCR and Human DNA Variations, Part 2," *Harlem DNA Lab*
January 16	HHMI Professional Development Workshop, "PCR and Human DNA Variations, Part 2," *Harlem DNA Lab*
January 22	Site visit by Hans Bosch and Laura Savini, WLIW, New York
January 23	*Saturday DNA!* "Express Yourself!" DNALC
January 29	Site visit by Marilyn Parks, Life Technologies Foundation, Carlsbad, California, and Randi Spatz, Invitrogen, Carlsbad, California
February 24	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
February 27	*Saturday DNA!* "Composting 101," DNALC
	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
March 3	Site visit to Carolina Biological Supply Company, Burlington, North Carolina
March 10	Oyster Bay Rotary Club Meeting Luncheon presenting DNALC programs and events, Oyster Bay, New York
March 12	*G2C Online* Workshop, Santa Clara University, Santa Clara, California
March 13	NIH *Inside Cancer* Workshop, Santa Clara University, Santa Clara, California
March 17	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
March 20	*Saturday DNA!* "Explore the Realm of Your DNA," DNALC
	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
March 20–21	National Science Teachers Association National Conference, "Exploring Gene Function in *C. elegans*: Mutations and RNA Interference," "Genomic Science," and "Silencing Genomes," Philadelphia, Pennsylvania
March 24–26	Joint Genome Institute User Meeting, Walnut Creek, California
March 26	*G2C Online* Workshop, Oklahoma City Community College, Oklahoma City
March 27	NIH *Inside Cancer* Workshop, Oklahoma City Community College, Oklahoma City
April 7	HHMI Professional Development Workshop, "Bacterial Transformation and Protein Isolation," *Harlem DNA Lab*
April 9	Site visit by graduate students from Molloy College, Rockville Centre, New York
April 10	HHMI Professional Development Workshop, "Bacterial Transformation and Protein Isolation," *Harlem DNA Lab*
April 11–14	NIH SEPA Principal Investigators Meeting, Birmingham, Alabama
April 13	*Great Moments in DNA Science* Honors Seminar: "Dead End? Not Anymore! Molecular Targeted Therapy for Skin Cancer," Dr. Chih-Shan Jason Chen, Memorial Sloan-Kettering Cancer Center, New York
April 16	*G2C Online* Workshop, iBio Institute, Harold Washington College, Chicago, Illinois
April 17	NIH *Inside Cancer* Workshop, iBio Institute, Harold Washington College, Chicago, Illinois
April 17	*Saturday DNA!* "The Insomniac's Guide to Wildlife," DNALC
April 20	Site visit by Debby Hirshman, Victor Centers for Jewish Genetic Diseases, Philadelphia, Pennsylvania
April 21	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
April 23	CSHL Association Directors, viewing of "Naturally Obsessed: The Making of a Scientist," DNALC
April 23–24	NSF *iPlant Collaborative Genomics in Education* Workshop, Spelman College, Atlanta, Georgia
April 24	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
April 26	Site visit by Kevin Shine, Verizon Communications, New York
April 29	*G2C Online* Workshop, Biogen Idec, Cambridge, Massachusetts
	Great Moments in DNA Science Honors Seminar: "The Role of Wnt/β-catenin Antagonist chibby in Lung Development," Dr. Damon Love, Weill Cornell College of Medicine, New York
	Site visit by Jackie Dorrance, Beckman Foundation, Irvine, California
	Site visit by Robert Isaksen and Lorraine Aycock, Bank of America Long Island, Melville, New York
	Site visit by Judy Calabrese and Maureen Laness, CSHL Directors, *Harlem DNA Lab*
April 30	NIH *Inside Cancer* Workshop, Biogen Idec, Cambridge, Massachusetts
May 3	Meeting with Minister of Higher Education, Sheikh Nahyan binMubarak Al Nahyan, Abu Dhubai, United Arab Emirates
May 7–8	NSF *iPlant Collaborative Genomics in Education* Workshop, University of Texas, Austin
May 8	HHMI Professional Development Workshop "DNA Isolation, Inheritance, and Variability," *Harlem DNA Lab*
May 12	Site visit by Roland Jimenez, Community Bank, Hauppauge, New York
May 14–15	HHMI Professional Development Workshop, "PCR, Part 1," *Harlem DNA Lab*
May 15	*Saturday DNA!* "Walking Whales and Genetic Tales," DNALC
May 17	Site visit by Srinivasa Rao, Indian Institute of Biotechnology, Hyderabad, India
May 18	*Great Moments in DNA Science* Honors Seminar: "Thioctans: A Novel Approach to Cancer Chemotherapy," Dr. Zuzana Zachar, Stony Brook University, New York
May 21	HHMI Professional Development Workshop, "PCR, Part 2," *Harlem DNA Lab*
	G2C Online Workshop, University of Colorado, Aurora

May 21–22	NSF *iPlant Collaborative Genomics in Education* Workshop, Lawrence Berkeley National Laboratory, Berkeley, California
May 22	NIH *Inside Cancer* Workshop, University of Colorado, Aurora
May 22	HHMI Professional Development Workshop, "PCR, Part 2," *Harlem DNA Lab*
May 22	2010 S.T.E.M. Expo sponsored by Morningside Area Alliance, "DNA Extraction," Harlem, New York
May 24–26	NSF *iPlant Collaborative* 2010 Conference, Las Vegas, Nevada
May 26	NSF *iPlant Collaborative, DNA Subway* Workshop, Las Vegas, Nevada
June 4	Site visit by Kidgie Williams, Hospitality Committee for United Nations Delegations, Inc., and United Nations delegates' family members, New York
June 4–5	NSF *Silencing Genomes* Follow-up Workshop, St. Louis Science Center, Missouri
June 6	World Science Festival, "Banana DNA Extraction," New York
June 8	17th Annual Golf Outing, Piping Rock Club, Locust Valley, New York
June 11–12	NSF *iPlant Collaborative Genomics in Education* Workshop, Howard University, Washington, D.C.
	NSF *Silencing Genomes* Follow-up Workshop, Rust College, Holly Springs, Mississippi
June 12	*Saturday DNA!* "Solved! The Mystery of Anastasia Romanov," DNALC
June 14–18	*DNA Science* Workshop, *Harlem DNA Lab*
	DNA Science Workshop, Trinity School, New York
	Fun with DNA Workshop, Trinity School, New York
June 17–18	NSF *Silencing Genomes* Follow-up Workshop, North Carolina Agricultural & Technical State University, Greensboro
June 18	Site visit by Mike Scanlon, Cornell University, Ithaca, New York
June 20–25	Milwaukee School of Engineering Workshop, SMART protein modeling training, New York
June 21–22	*Charter Membership High School Professional Development* Workshop, The Chapin School, "Human Genomics," New York
June 21–25	*Fun with DNA* Workshop, The Chapin School, New York
	Human Genomics Workshop, The Chapin School, New York
June 22	Site visit by Theresa Chilianis, Michael Lardner, and Phil Summers, MSG Varsity, Woodbury, New York
June 23–24	*Charter Membership Middle School Professional Development* Workshop, The Chapin School, "*Fun with DNA*," New York
June 24–25	NSF *Silencing Genomes* Follow-up Workshop, Pasadena City College, California
June 25–26	NSF *iPlant Collaborative Genomics in Education* Workshop, The Rockefeller University, New York
June 28	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
June 28–July 2	*DNA Science* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Fun with DNA Workshop, DNALC *West*
June 29	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
June 29–30	NSF *Silencing Genomes* Follow-up Workshop, Howard University, Washington, D.C.
June 30	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 1" *Harlem DNA Lab*
July 1	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
July 6	HHMI Professional Development Workshop, "DNA Structure and Isolation," *Harlem DNA Lab*
July 6–9	*Green Genes* Workshop, DNALC
	Plant Genomics Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
July 7	HHMI Professional Development Workshop, "Variability and Inheritance," *Harlem DNA Lab*
July 8	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
July 8–9	NSF *iPlant Collaborative Genomics in Education* Workshop, University of Chicago, Illinois
July 9	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
July 12	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
July 12–16	*DNA Science* Workshop, DNALC
	Human Genomics Workshop, DNALC
	World of Enzymes Workshop, DNALC
	DNA Science Workshop, DNALC *West*
July 13	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
July 14	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 1," *Harlem DNA Lab*
July 15	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
	Site visit by Suzi Lewis, University of California, Berkeley
July 16	HHMI Professional Development Workshop, "Genetically Modified Foods," *Harlem DNA Lab*
July 19–23	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	Silencing Genomes Workshop, DNALC
	Green Genes Workshop, DNALC *West*

July 19–30	HHMI New York Leadership Symposium, *Harlem DNA Lab*
July 20	Site visit by Rafael Palacios, Universidad Nacional Autónoma de México, Cuernavaca Center for Genomic Sciences, Cuernavaca, Mexico
July 26–30	*DNA Science* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Genetic Horizons Workshop, DNALC *West*
July 29	Site visit by Mort Slater, Gateway to Higher Education, New York, and Arthur Registre, Uniondale High School, New York
July 31	Botanical Society of America, *DNA Subway* Hands-on Workshop, Providence, Rhode Island
July 31–Aug. 4	American Society of Plant Biologists Annual Conference, *DNA Subway* Demonstrations and Poster, Montreal, Canada
August 2	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
August 2–6	*Fun with DNA* Workshop, DNALC
	Green Genes Workshop, DNALC
	Human Genomics Workshop, DNALC
	Fun with DNA Workshop, DNALC *West*
August 3	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
August 4	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 1," *Harlem DNA Lab*
August 5	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
August 6	HHMI Professional Development Workshop, "Genetically Modified Foods," *Harlem DNA Lab*
August 9	HHMI Professional Development Workshop, "DNA Transformation and Protein Isolation," *Harlem DNA Lab*
August 9–13	*DNA Science* Workshop, DNALC
	Genetic Horizons Workshop, DNALC
	World of Enzymes Workshop, DNALC
	World of Enzymes Workshop, DNALC *West*
August 10	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
August 11	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 1," *Harlem DNA Lab*
August 12	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
August 16–20	*Forensic Detectives* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	Green Genes Workshop, DNALC
	DNA Science Workshop, DNALC *West*
	DNA Science Workshop, Mentors in Medicine, *Harlem DNA Lab*
August 17	Site visit by Esther Baena, Harvard Medical School, Cambridge, Massachusetts
August 18	Site visit by Ted Scovell and Mark Stoeckle, The Rockefeller University, New York
August 19–20	NSF *Silencing Genomes* Follow-up Workshop, Houston Community College, Texas
August 23–27	*DNA Science* Workshop, DNALC
	Fun with DNA Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Human Genomics Workshop, DNALC *West*
Aug. 30–Sept. 3	*DNA Science* Workshop, DNALC
	Green Genes Workshop, DNALC
	World of Enzymes Workshop, DNALC
	Green Genes Workshop, DNALC *West*
September 2	Site visit by Pauline Charman, University of Western Australia, Perth
September 14	Eugenics Exhibit, Holocaust Memorial and Tolerance Center, Glen Cove, New York
September 15	Site visit by Torbert Rocheford, Purdue University, West Lafayette, Indiana
September 16–17	NSF *iPlant Collaborative Genomics in Education* Workshop, University of Washington, Seattle
September 17	Site visit by Anne and Walter Meier, The Robertson Foundation for Government, Juno Beach, Florida
September 25	HHMI Professional Development Workshop, "DNA Structure and Isolation, Part 2," *Harlem DNA Lab*
October 5	Site visit by Denise Smith, Vincent Torti, and Rick Garrett, Saint Dominic's Church and Schools, Oyster Bay, New York
October 15	HHMI Professional Development Workshop, "Inheritance and Variability," *Harlem DNA Lab*
October 16	HHMI Professional Development Workshop, "Inheritance and Variability," *Harlem DNA Lab*
	New York Academy of Sciences Graduate Training, New York
	Saturday DNA! "Classify Me!" DNALC
October 18–19	NIH SEPA Grant Review, Bethesda, Maryland
October 22	HHMI Professional Development Workshop, "Bacterial Transformation and Protein Isolation," *Harlem DNA Lab*
October 23	HHMI Professional Development Workshop, "Bacterial Transformation and Protein Isolation," *Harlem DNA Lab*
November 2–3	NSF *iPlant Collaborative Genomics in Education* Workshop, University of Minnesota, Saint Paul
November 3–6	National Association of Biology Teachers Conference, "Orphaned Data," "Frontiers in Genomics," "DNA

	Subway," "RNAi/CBSC," "Iceman," "CSI," "LabCenter," Minneapolis, Minnesota
November 4	Site visit by Paula Olsiewski, Alfred P. Sloan Foundation, *Harlem DNA Lab*
November 5	Site visit by Verizon Fios One MYLITV for filming
November 8	*iPlant Collaborative* NSF site visit, Tucson, Arizona
November 8–9	Science Teachers Association of New York State Conference, presentations on DNALC family of websites, Rochester, New York
November 12	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
November 13	HHMI Professional Development Workshop, "DNA Analysis and Forensics," *Harlem DNA Lab*
	Saturday DNA! "DNA Barcoding," DNALC
November 22	Baruch College, "*iPlant Collaborative, DNA Subway*," New York
	Marcus Foundation "Gene Screen," Victor Centers for Jewish Genetic Diseases, Albert Einstein Medical Center, Philadelphia, Pennsylvania
Nov. 29–Dec. 11	Singapore Primary Teachers Attachment, DNALC
December 3	Site visit by Mohammad Alshehri, King Saud University, and Talal Al-Malki, Taif University, Saudi Arabia
	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 1," *Harlem DNA Lab*
December 4	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 1," *Harlem DNA Lab*
December 9	Site visit by Suzanne Sunshine, S. Sunshine & Associates, New York
December 11	HHMI Professional Development Workshop, "PCR and Human DNA Variation, Part 2," *Harlem DNA Lab*
	Saturday DNA! "What Does Your Nose Know?" DNALC
December 14–15	Site visit by Steve Goff and Eric Lyons, *iPlant Collaborative*, Tucson, Arizona
December 15–16	NSF *iPlant Collaborative Genomics in Education* Workshop, University of Texas, Brownsville and Texas Southmost College, Brownsville
December 16	Site visit by James Jorasch and Megan Kingery, Science House, *Harlem DNA Lab*

Sites of Major Faculty Workshops 1985–2010

Key: *Middle School*   High School   **College**

ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	University of Alaska, Fairbanks	1996
ARIZONA	Arizona State University, Tempe	2009
	Tuba City High School	1988
ARKANSAS	Henderson State University, Arkadelphia	1992
CALIFORNIA	**California State University, Dominguez Hills**	**2009**
	California State University, Fullerton	**2000**
	California Institute of Technology, Pasadena	**2007**
	Cañada College, Redwood City	1997
	City College of San Francisco	2006
	Contra Costa County Office of Education, Pleasant Hill	2002, 2009
	Foothill College, Los Altos Hills	**1997**
	Harbor–UCLA Research & Education Institute, Torrance	2003
	Los Angeles Biomedical Research Institute (LA Biomed), Torrance	**2006**
	Laney College, Oakland	1999
	Lutheran University, Thousand Oaks	1999
	Oxnard Community College, Oxnard	2009
	Pasadena City College, Pasadena	**2010**
	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001, 2008
	San Francisco State University	**1991**
	San Jose State University	**2005**
	Santa Clara University	2010
	University of California, Berkeley	**2010**
	University of California, Davis	1986
	University of California, Northridge	**1993**
COLORADO	Aspen Science Center	2006
	Colorado College, Colorado Springs	1994, 2007
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998, 2009, 2010
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
FLORIDA	Armwood Senior High School, Tampa	1991
	Florida Agricultural & Mechanical University, Tallahassee	2007–2008
	North Miami Beach Senior High School	1991
	University of Miami School of Medicine	2000
	University of Western Florida, Pensacola	1991
GEORGIA	Fernbank Science Center, Atlanta	1989, 2007
	Morehouse College, Atlanta	**1991, 1996, 1997**
	Spelman College, Atlanta	**2010**
HAWAII	Kamehameha Secondary School, Honolulu	1990
IDAHO	University of Idaho, Moscow	1994
ILLINOIS	Argonne National Laboratory	1986–1987
	iBio Institute/Harold Washington College, Chicago	2010
	Illinois Institute of Technology, Chicago	2009
	University of Chicago	**1992, 1997, 2010**
INDIANA	Butler University, Indianapolis	1987
IOWA	Drake University, Des Moines	1987
KANSAS	University of Kansas, Lawrence	1995
KENTUCKY	Murray State University	1988
	University of Kentucky, Lexington	1992
	Western Kentucky University, Bowling Green	1992
LOUISIANA	Bossier Parish Community College	2009
	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
	Foundation for Blood Research, Scarborough	2002

MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	National Center for Biotechnology Information, Bethesda	2002
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
MASSACHUSETTS	Beverly High School	1986
	Biogen Idec, Cambridge	2002, 2010
	Boston University	**1994, 1996**
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	The Winsor School, Boston	1987
	Whitehead Institute for Biomedical Research, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
MINNESOTA	Minneapolis Community and Technical College	2009
	University of Minnesota, St. Paul	2005
	University of Minnesota, St. Paul	**2010**
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990–1991
	Rust College, Holly Springs	**2006–2008, 2010**
MISSOURI	**St. Louis Science Center, St. Louis**	**2008–2009, 2010**
	Stowers Institute for Medical Research, Kansas City	2002, 2008
	Washington University, St. Louis	**1989, 1997**
NEVADA	University of Nevada, Reno	1992
NEW HAMPSHIRE	Great Bay Community College, Portsmouth	2009
	New Hampshire Community Technical College, Portsmouth	**1999**
	St. Paul's School, Concord	1986–1987
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
	Raritan Valley Community College, Somerville	2009
NEW MEXICO	Biolink Southwest Regional Meeting, Albuquerque	2008
NEW YORK	Albany High School	1987
	American Museum of Natural History	2007
	Bronx High School of Science	1987
	Canisius College, Buffalo	2007
	Cold Spring Harbor High School	1985, 1987
	Columbia University	**1993**
	Cornell University, Ithaca	**2005**
	DeWitt Middle School, Ithaca	*1991, 1993*
	Dolan DNA Learning Center	1988–1995, 2001–2004, 2006–2009
	Dolan DNA Learning Center	**1990, 1992, 1995, 2000**
	Dolan DNA Learning Center	*1990–1992*
	DNALC *West*	2005
	Fostertown School, Newburgh	*1991*
	Harlem DNA Lab, East Harlem	2008–2009
	Huntington High School	1986
	Irvington High School	1986
	John Jay College of Criminal Justice	2009
	Junior High School 263, Brooklyn	*1991*
	Lindenhurst Junior High School	*1991*
	Mt. Sinai School of Medicine	1997
	New York City Department of Education	2007
	New York Institute of Technology	2006
	New York Institute of Technology	**2006**
	Orchard Park Junior High School	*1991*
	Plainview–Old Bethpage Middle School	*1991*

	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Stuyvesant High School	1998–1999
	The Rockefeller University	2003
	The Rockefeller University	**2010**
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Lake Saranac	2001
	Union College, Schenectady	2004
	United States Military Academy, West Point	**1996**
	Wheatley School, Old Westbury	1985
NORTH CAROLINA	CIIT Center for Health Research, Triangle Park	2003
	North Carolina Agricultural & Technical State University, Greensboro	**2006–2007, 2009–2010**
	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	Langston University, Langston	2008
	North Westerville High School	1990
OKLAHOMA	Tulsa Community College, Tulsa	2009
	Oklahoma City Community College	**2000**
	Oklahoma City Community College	2006–2007, 2010
	Oklahoma Medical Research Foundation, Oklahoma City	2001
	Oklahoma School of Science and Math, Oklahoma City	1994
OREGON	Kaiser Permanente-Center for Health Research, Portland	2003
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
	Kimmel Cancer Center, Philadelphia	2008
RHODE ISLAND	Botanical Society of America, Providence	2010
SOUTH CAROLINA	**Clemson University, Clemson**	**2004**
	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	**1988**
TENNESSEE	NABT Professional Development Conference, Memphis	2008
TEXAS	Austin Community College–Rio Grande Campus	2000
	Austin Community College–Eastview Campus	**2007–2009**
	Houston Community College Northwest, Houston	**2009, 2010**
	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Midland College, Midland	2008
	Southwest Foundation for Biomedical Research, San Antonio	2002
	Taft High School, San Antonio	1991
	Texas A&M, AG Research and Extension Center, Weslaco	2007
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999, 2004, 2010**
	University of Texas, Brownsville	**2010**
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998, 2000**
	Utah Valley State College, Orem	**2007**
VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	Jefferson School of Science, Alexandria	1987
	Mathematics and Science Center, Richmond	1990
	Mills Godwin Specialty Center, Richmond	1998
	Virginia Polytechnic Institute and State University, Blacksburg	**2005, 2008–2009**
WASHINGTON	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001, 2008
	University of Washington, Seattle	**1993, 1998, 2010**
WASHINGTON, D.C	**Howard University**	**1992, 1996, 2009–2010**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003

	Madison Area Technical College	**1999, 2009**
	Marquette University, Milwaukee	1986–1987
	University of Wisconsin, Madison	1988, 1989
	University of Wisconsin, Madison	**2004**
WYOMING	University of Wyoming, Laramie	1991
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994

AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
AUSTRIA	**Vienna Open Lab**	**2007**
CANADA	Red River Community College, Winnipeg, Manitoba	1989
CHINA	**Ho Yu College, Hong Kong**	**2009**
GERMANY	Urania Science Center, Berlin	2008
ITALY	Porto Conte Research and Training Laboratories, Alghero	1993
	International Institute of Genetics and Biophysics, Naples	1996
MEXICO	ASPB Plant Biology, Merida	2008
PANAMA	**University of Panama, Panama City**	**1994**
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001–2005
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University, Uppsala	2000
THE NETHERLANDS	**International Chromosome Conference, Amsterdam**	**2007**

2010 PRESS PUBLICATIONS

Serials

Genes & Development, Vol. 24 (www.genesdev.org)
Genome Research, Vol. 20 (www.genome.org)
Learning & Memory, Vol. 17 (www.learnmem.org)
RNA, Vol. 16 (www.rnajournal.org)
Cold Spring Harbor Symposia on Quantitative Biology, Vol. 74:
    Evolution: The Molecular Landscape, edited by Bruce
    Stillman, David Stewart, and Jan Witkowski
Cold Spring Harbor Protocols (www.cshprotocols.org)
Cold Spring Harbor Perspectives in Biology
    (www.cshperspectives.org)

Laboratory Manuals

Emerging Model Organisms: A Laboratory Manual, Volume 2
Drosophila *Neurobiology: A Laboratory Manual,* edited by
    Bing Zhang, Marc R. Freeman, and Scott Waddell
Imaging: A Laboratory Manual, edited by Rafael Yuste
Imaging in Developmental Biology: A Laboratory Manual,
    edited by James Sharpe and Rachel O. Wong; Series
    Editor Rafael Yuste
RNA: A Laboratory Manual, by Donald C. Rio, Manuel Ares Jr.,
    Gregory J. Hannon, and Timothy W. Nilsen

Handbooks

At the Helm: Leading Your Laboratory, Second Edition, by Kathy
    Barker
Career Opportunities in Clinical Drug Research, by Rebecca J.
    Anderson

Advanced Textbooks

Guide to the Human Genome, by Stewart Scherer (online and/or
    print)
Means to an End: Apoptosis and Other Cell Death Mechanisms, by
    Douglas R. Green
RNA Worlds: From Life's Origins to Diversity in Gene Regulation,
    by John F. Atkins, Raymond F. Gesteland, and Thomas R.
    Cech

Monographs (*Perspectives in Biology Topic Collections*)

Generation and Interpretation of Morphogen Gradients, edited by
    James Briscoe, Peter A. Lawrence, and Jean-Paul Vincent
Cell–Cell Junctions, edited by W. James Nelson and Elaine Fuchs
Symmetry Breaking in Biology, edited by Rong Li and Bruce
    Bowerman
*NF-κB: A Network Hub Controlling Immunity, Inflammation,
    and Cancer,* edited by Michael Karin and Louis M. Staudt
The Origins of Life, edited by David Deamer and Jack W. Szostak
The p53 Family, edited by Arnold J. Levine and David P. Lane
The Nucleus, edited by Tom Misteli and David L. Spector
Auxin Signaling: From Synthesis to Systems Biology, edited by
    Mark Estelle, Dolf Weijers, Karin Ljung, and
    Ottoline Leyser
Neuronal Guidance: The Biology of Brain Wiring, edited by Marc
    Tessier-Lavigne and Alex L. Kolodkin
Immunoreceptor Signaling, edited by Lawrence E. Samelson and
    Andrey Shaw
Cell Biology of Bacteria, edited by Lucy Shapiro and Richard M.
    Losick

Science and Society

Speaking of Genetics: A Collection of Interviews, by Jane Gitschier
Sydney Brenner: A Biography, by Errol C. Friedberg
The Honest Look, by Jennifer L. Rohn

Other

Neurobiology of the Leech, edited by Kenneth J. Muller, John G.
    Nicholls, and Gunther S. Stent (reissue of 1981 title)
CSHL Annual Report 2009, Yearbook Edition
CSHL Annual Report 2009
Banbury Center Annual Report 2009
Watson School of Biological Sciences Annual Report 2009

Websites

http://cshmonographs.org/index.php/monographs; Cold Spring
    Harbor Monographs Archive Online
http://www.cshl.symposium.org; Symposia, Vol. 74 added to
    online Symposia website
http://www.humangenomeguide.org

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

Cold Spring Harbor Laboratory's research activities are complemented by the creation of journals, books, and electronic media that extend the professional education of scientists around the world. With a tradition of excellence originating in the 1930s, Cold Spring Harbor Laboratory Press is continuing to redefine its role as a publisher in the digital age. Although distribution channels for scientific information continue to evolve and diversify, the mission of the Press remains to identify innovative research, technologies, and scientists, to amplify their effectiveness by selecting, aggregating, and curating information that research communities can use, and to deliver the information when, where, and how the communities want it. The task is complex and in 2010, there were significant achievements. More than 10 million research articles were downloaded internationally from our journals, over 3000 electronic subscriptions were delivered to academic institutions worldwide, and 35,000 copies of print books were shipped from our warehouses in the United States and Europe. The publication program encompasses six research journals, two of them among the most highly ranked in biology; authoritative laboratory manuals; and best-selling primers on professional skill development for scientists. Their distribution makes a significant contribution to the Laboratory's broad educational mission and strengthens Cold Spring Harbor's association with excellence.

Financially, the established journals performed strongly in 2010. The start-up costs of a new journal were written off. Sales of print books were weakened by lower backlist orders and delayed publication of some new titles. To improve the book program's future financial performance, substantial expense savings were made through procedural changes and by staff reduction at the year's end.

Journals

Journal publishing remains the largest contributor of revenue and is vital to the financial sustainability of the publishing program. Journal subscriptions did well in what continued to be a challenging market, as academic libraries continued to come to terms with reduced budgets. Nevertheless, total subscription revenue for all five established journal titles increased by more than 5%. Despite continued merger activity within the laboratory supplies sector that reduces the potential client base, advertising revenue across the journals met its goals, with a continued but still gradual shift in the relative proportion of income from print and online sales.

Genes & Development completed a satisfactory year with, for the first time, a single center of editorial operations that provided authors with faster and more consistent decisions. Both *Genes & Development* and *Genome Research* continued to rank among the most important publications in genetics and cell and developmental biology, attracting more submissions, maintaining impressive impact factors, and, through article downloads, finding intense use in academic centers worldwide. *RNA* maintained its status as the premier specialist journal in the burgeoning field of RNA biology and a new 5-year publishing contract was signed with its owner, The RNA Society. *Learning & Memory* remains a source of specialized work in the molecular analysis of nervous system plasticity.

The subscription base of *Cold Spring Harbor Protocols* made solid gains and its usage continued to grow. The new journal *Cold Spring Harbor Perspectives in Biology*, with content of exceptional quality, found remarkable usage during an 18-month free trial period, a result that augurs well for subscription growth. The journal's unique feature is the publication of collections of articles that have each been commissioned to provide an in-depth, coherent survey of a single topic. A large number of subject collections from cell and molecular biology are in the publication pipeline, and each collection has commissioning editors with outstanding international reputations. Each article
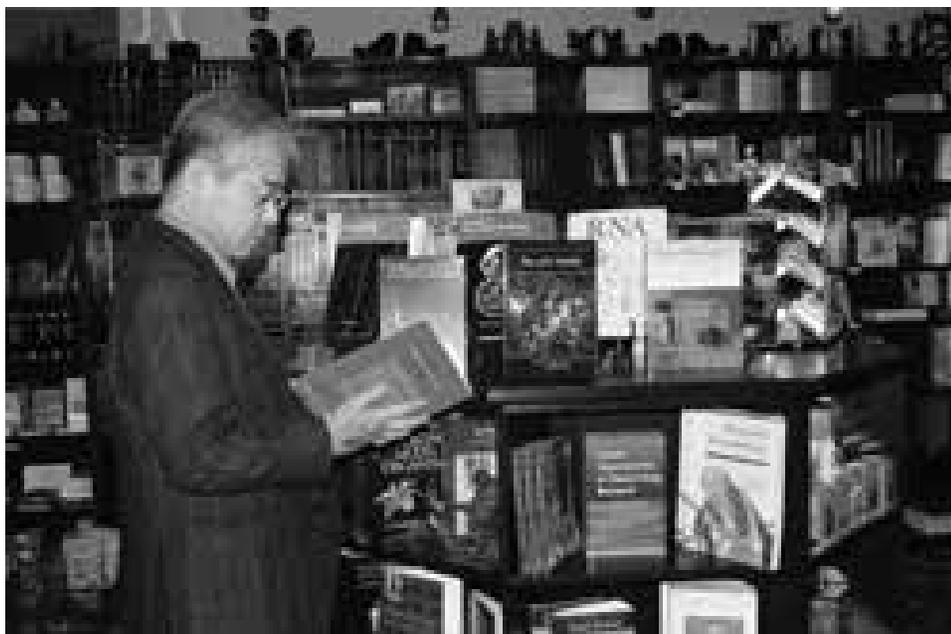
Cold Spring Harbor Laboratory bookstore

is being published individually in an issue of the journal, but when complete, the collections will be available separately. The response from scientists to the *Perspectives* concept has been so positive that a sister publication, *Cold Spring Harbor Perspectives in Medicine,* is being planned as a source of research information for physicians and scientists working in cancer, neuropathies, psychiatric illness, infectious disease, metabolic syndrome, and other disorders.

The type of content in both *Perspectives* and *Protocols* was previously published only in printed book form. In a digital journal context, it is now discoverable in biomedical science's most important bibliographic database, PubMed, enhancing its prestige and value to the research community. Nevertheless, the content is still publishable in print as topic-specific books. Of the 26 books published by the Press in 2010, 12 originated in the *Perspectives* journal and can be printed on demand from online content. In this way, unpredictable markets for print publications can be served with information that also has a digital, subscription-based business model.

A multichannel approach to content distribution is also seen in the *Guide to the Human Genome*, a unique, richly linked online resource that is structured like a book, but published online as well as in print. Online users can use search tools to explore the human genome through direct links to the reference sequence and compare human sequences with those from other species. The Guide's low-cost hosting, an ordering system allowing individual and institutional customers to purchase and immediately access the online content, and print-on-demand capability together may provide a publishing solution for future Press titles with both print and online markets.

Other Electronic Publications

Many forms of publishing have been transformed in the past 2 years by the explosion in sales of handheld devices like smartphones, tablet computers such as the iPad, and e-readers like the Kindle. The use of software applications on these devices for information access and reuse is growing exponentially. Like all publishers, the Press is closely monitoring these changes and the choices being made by the consumers of information, but we remain cautious about investing in these avenues

for the distribution of our content. A limited number of our book titles are available for the Kindle reader on a case-by-case basis, and sales, predictably, were low while the device remained expensive. But with the availability of new models at lower prices, and now that Kindle software can run on many different devices, there may be a larger audience in the future. There is no doubt that Cold Spring Harbor's book content must find its way into digital media and our biographies, histories, and novels will find part of their audience on digital reading devices. But these are a small part of our output, and much thought, discussion, and exploration are being devoted to the more important consideration of what working professionals need and the choices of digital media they will make. The experience of producing and marketing electronic books has been valuable and so has the creation of an iPhone application for our journal *Genome Research* that allows abstracts of papers to be read everywhere and full text to be accessed when the phone is connected to a subscribing institution's network. Our goal remains to make our content available in as many ways as readers require, and we will continue to explore the options available.

Books in Print

The year's crop of new books included five manuals of techniques, a traditionally strong component of our publishing program. Molecular biology has been transformed by the discovery of new species of RNA molecule that regulate gene function in vivo and in vitro and *RNA: A Laboratory Manual* is a uniquely authoritative source of expertise in working with these molecules. Imaging technologies are also transforming the way experimental biology is done, and two new manuals provided instruction in both general and more specific approaches to the use of these methods.

Our widely praised list of professional development handbooks was enhanced by a new book about career opportunities in industry and the second edition of *At the Helm,* Kathy Barker's enormously successful source of advice and counsel for principal investigators.

As knowledge in biology becomes denser and more detailed, it is harder to write short, informative, thought-provoking books on specific topics. Yet Douglas Green has achieved this distinction with *Means to an End*, a personal account of how cells die that has been widely praised. Inspiration for young scientists of a different but still potent kind is found in Errol Friedberg's account of the notable life and work of Sydney Brenner and in a collection of insightful interviews conducted by Jane Gitschier with a variety of people in and around science (*Speaking of Genetics*). And in *The Honest Look*, a second novel by the cell biologist Jennifer Rohn, readers can find a human drama based in a realistic portrayal of the scientific enterprise and the men and women engaged in it. A complete list of the year's new titles can be found at the beginning of this report.

The year's best-sellers included *Molecular Cloning, At the Bench, Symposium 74: Evolution, Statistics at the Bench, Epigenetics, Live Cell Imaging, At the Helm*; and *RNA: A Laboratory Manual.* New translation contracts included Japanese translations of *Statistics at the Bench* and *At the Helm*; and Chinese translations of *Live Cell Imaging* and *Transcriptional Regulation in Eukaryotes.*

The generation of book sales worldwide continues to rely heavily on a monthly electronic newsletter. Our websites continue to be cornerstones of our presence in the information landscape and our long relationship with Amazon remains essential to the sale of our books in the United States. Internationally, we benefit greatly from the local expertise of commission-based sales agents in Germany and several associated European countries, China, Japan, India, and South America. Warehousing, order processing, and book distribution outside the United States continue to be handled by a company in the United Kingdom.

Staff

During the year, we said farewell to Ingrid Benirschke, Ann Boyle, Mary Cozza, Joan Ebert, Maria Fairchild, Lauren Heller, Kaaren Kockenmeister, Kathryn LaForgia, Christina Lo, Katherine MacDon-

John R. Inglis
Executive Director
and Publisher

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2010
(with comparative financial information as of December 31, 2009)

	2010	2009
Assets:		
Cash and cash equivalents	$ 60,766,362	65,950,558
Grants receivable	7,725,659	6,200,398
Contributions receivable, net	104,272,591	119,740,185
Publications inventory	3,555,193	4,570,278
Investments	276,424,730	241,595,564
Restricted use assets	2,580,471	2,111,090
Other assets	9,922,097	10,398,213
Land, buildings, and equipment, net	238,777,879	237,791,406
Total assets	$ 704,024,982	688,357,692
Liabilities and Net Assets:		
Liabilities:		
Accounts payable and accrued expenses	$ 25,635,009	26,475,011
Deferred revenue	8,210,668	5,640,029
Bonds payable	97,200,000	97,200,000
Total liabilities	131,045,677	129,315,040
Net assets:		
Unrestricted	227,854,292	235,125,827
Temporarily restricted	242,589,902	142,357,714
Permanently restricted	102,535,111	181,559,111
Total net assets	572,979,305	559,042,652
Total liabilities and net assets	$ 704,024,982	688,357,692

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2010
(with summarized financial information for the year ended December 31, 2009)

	Unrestricted	Temporarily Restricted	Permanently Restricted	2010 Total	2009 Total
Revenue and other support:					
Public support–contributions and non-federal grant awards	$ 13,624,936	30,685,893	1,109,499	45,420,328	67,103,796
Federal grant awards	35,405,944	–	–	35,405,944	29,648,968
Indirect cost allowances	25,973,354	–	–	25,973,354	21,016,585
Investment return utilized	18,309,184	–	–	18,309,184	18,216,748
Program fees	6,971,358	–	–	6,971,358	5,484,810
Publications sales	9,251,441	–	–	9,251,441	9,136,188
Dining services	4,319,847	–	–	4,319,847	4,103,820
Rooms and apartments	3,556,328	–	–	3,556,328	3,255,480
Miscellaneous	2,126,513	–	–	2,126,513	1,962,873
Net assets released from restrictions	34,646,285	(34,646,285)	–	–	–
Total revenue and other support	154,185,190	(3,960,392)	1,109,499	151,334,297	159,929,268
Expenses:					
Research	86,810,119	–	–	86,810,119	74,005,657
Educational programs	17,121,875	–	–	17,121,875	14,964,908
Publications	10,877,055	–	–	10,877,055	9,032,225
Banbury Center conferences	1,370,778	–	–	1,370,778	1,069,044
Dolan DNA Learning Center programs	1,406,339	–	–	1,406,339	1,467,947
Watson School of Biological Sciences programs	3,535,758	–	–	3,535,758	3,595,810
General and administrative	14,746,870	–	–	14,746,870	13,903,883
Dining services	5,137,648	–	–	5,137,648	4,712,901
Total expenses	141,006,442	–	–	141,006,442	122,752,375
Excess (deficiency) of revenue and other support over (under) expenses	13,178,748	(3,960,392)	1,109,499	10,327,855	37,176,893
Other changes in net assets:					
Investment (loss) return excluding amount utilized	(428,497)	9,028,095	–	8,599,598	24,463,751
Change in fair value of interest-rate swap	(4,990,800)	–	–	(4,990,800)	24,524,899
Release of temporarily restricted capital funds	4,527,558	(4,527,558)	–	–	–
Net asset reclassification based on change in law/interpretation	(19,558,544)	99,692,043	(80,133,499)	–	–
(Decrease) increase in net assets	(7,271,535)	100,232,188	(79,024,000)	13,936,653	86,165,543
Net assets at beginning of year	235,125,827	142,357,714	181,559,111	559,042,652	472,877,109
Net assets at end of year	$ 227,854,292	242,589,902	102,535,111	572,979,305	559,042,652

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2010
(with comparative financial information for the year ended December 31, 2009)

	2010	2009
Cash flows from operating activities:		
Increase in net assets	$ 13,936,653	86,165,543
Adjustments to reconcile increase in net assets		
to net cash provided by operating activities:		
Change in fair value of interest rate swap	4,990,800	(24,524,897)
Depreciation and amortization	15,443,523	9,282,371
Net appreciation in fair value of investments	(23,724,939)	(36,992,017)
Contributions restricted for long-term investment	(1,712,498)	(9,121,400)
Changes in assets and liabilities:		
Grants receivable	(1,525,261)	2,397,001
Contributions receivable, net of financing activities	11,309,672	(18,458,455)
Publications inventory	1,015,085	(644,311)
Other assets	929,237	(611,026)
Restricted use assets	(469,381)	1,456,592
Accounts payable and accrued expenses, net of financing activities	(2,521,650)	32,680
Deferred revenue	2,570,639	1,678,029
Net cash provided by operating activities	20,241,880	10,660,110
Cash flows from investing activities:		
Capital expenditures	(16,429,996)	(48,389,574)
Proceeds from sales and maturities of investments	36,404,485	140,454,706
Purchases of investments	(47,508,712)	(131,634,094)
Net change in investment in employee residences	(453,121)	440,775
Net cash used in investing activities	(27,987,344)	(39,128,187)
Cash flows from financing activities:		
Permanently restricted contributions	1,109,499	2,255,040
Contributions restricted for investment in land, buildings, and equipment	602,999	6,866,360
Decrease in contributions receivable	4,157,922	3,399,927
Decrease in deposits with bond trustee	–	11,886,744
(Decrease) increase in accounts payable relating to capital expenditures	(3,309,152)	403,725
Decrease in deposits with swap counterparty	–	17,380,247
Net cash provided by financing activities	2,561,268	42,192,043
Net (decrease) increase in cash and cash equivalents	(5,184,196)	13,723,966
Cash and cash equivalents at beginning of year	65,950,558	52,226,592
Cash and cash equivalents at end of year	$ 60,766,362	65,950,558
Supplemental disclosure:		
Interest paid	$ 3,652,690	3,180,748
Noncash investing and financing activities:		
Contributed property	$ 57,475	98,085

FINANCIAL SUPPORT OF THE LABORATORY

Cold Spring Harbor Laboratory, Banbury Center, and the Dolan DNA Learning Center receive a substantial portion of their funding through grants from the federal government and through grants, capital gifts, and annual contributions from New York State, private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2010.

GRANTS January 1–December 31, 2010

COLD SPRING HARBOR LABORATORY

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
FEDERAL GRANTS				
NATIONAL INSTITUTES OF HEALTH				
Equipment Support	Dr. Pappin	05/13/10	05/12/11	$ 851,656 *[2]
Program Project and Center Support	Drs. Hannon/Krainer/Lowe/Spector/Stillman	01/01/07	12/31/11	4,743,330
	Dr. Stillman–Cancer Center Core	08/15/05	07/31/11	4,236,444
	Dr. Stillman–Cancer Center Core	09/30/09	03/31/11	59,081 [2]
	Dr. Zador–Faculty Recruitment	09/30/09	08/31/11	538,683 [2]
Cooperative Research Agreement Support[3]	Drs. Gingeras/Hannon	09/27/07	06/30/11	1,899,018
	Dr. Huang	09/08/06	08/31/11	201,418
	Drs. Lowe/Hannon/Hicks/Powers	09/01/10	08/31/11	871,519 *
Contract Support	Drs. Hannon/Lowe	12/31/09	12/31/11	999,999
Research Support	Dr. Churchland	09/01/10	08/31/13	248,999 *
	Dr. Dubnau	09/15/09	08/31/14	430,650
	Dr. Furukawa	03/01/10	02/28/15	435,000 *
	Dr. Hannon	09/01/09	08/31/13	353,133
	Drs. Hannon/Gingeras/McCombie	09/30/09	08/31/11	1,506,827 [2]
	Drs. Hannon/Gingeras/McCombie	09/30/09	08/31/11	1,103,288 [2]
	Drs. Hannon/Gingeras/McCombie	09/30/09	08/31/11	1,491,492 [2]
	Dr. Huang	04/16/09	04/30/11	210,000
	Dr. Huang	09/30/09	08/31/11	513,667 [2]
	Dr. Joshua-Tor	07/01/07	06/30/12	307,561
	Dr. Joshua-Tor	02/01/06	01/31/11	105,597
	Dr. Koulakov	02/01/08	01/31/12	415,800
	Drs. Koulakov/Enikolopov	07/15/10	04/30/14	348,000 *
	Dr. Krainer	09/21/07	08/31/11	656,937
	Dr. Li	07/01/10	03/31/15	526,630 *
	Dr. Lowe	09/15/09	08/31/14	353,133
	Dr. Martienssen	08/01/07	07/31/11	419,760
	Dr. Martienssen	09/29/09	08/31/11	348,000 [2]
	Dr. McCombie	07/23/10	04/30/15	1,135,298 *
	Dr. Mills	12/26/07	11/30/12	359,805
	Dr. Mitra	09/30/09	05/31/14	648,174
	Dr. Mitra	09/30/09	08/31/11	499,997 [2]

[1]Includes direct and indirect costs
[2]Award issued under the American Recovery and Reinvestment Act of 2009
[3]Cooperative research agreement funding amounts include only CSHL portion of award
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
	Dr. Mitra	08/01/10	05/31/13	$ 399,317 *
	Dr. Muthuswamy	02/01/09	12/31/13	389,393
	Drs. Powers/Lowe/Krasnitz/Sordella	09/29/09	08/31/11	2,374,043 [2]
	Dr. Powers	12/02/06	11/30/11	456,045
	Dr. D. Spector	04/01/90	03/31/11	676,357
	Dr. Stenlund	02/01/08	01/31/12	430,650
	Dr. Stillman	06/01/08	05/31/12	644,890
	Dr. Tonks	07/01/10	04/30/15	712,640 *
	Dr. Tonks	01/11/10	12/31/13	408,900 *
	Dr. Trotman	04/01/10	01/31/14	288,840 *
	Dr. Turner	07/15/10	06/30/15	361,000 *
	Dr. Van Aelst	07/01/08	03/31/13	391,500
	Dr. Van Aelst	01/01/09	12/31/13	361,050
	Drs. Wigler/McCombie	09/30/09	08/31/11	1,395,339 [2]
	Dr. Zador	09/18/08	07/31/13	366,053
	Dr. Zador	09/24/09	08/31/11	93,017 [2]
	Dr. Zador	09/27/10	05/31/15	435,000 *
	Dr. Zhong	06/01/10	05/31/13	348,000 *
Research Subcontracts				
NIH/Allen Institute for Brain Science Consortium Agreement	Dr. Mitra	09/15/09	08/31/11	229,681
NIH/Angion Biomedica Corp. Consortium Agreement	Dr. Enikolopov	09/30/09	08/31/11	278,368
NIH/Brookhaven National Laboratory Consortium Agreement	Dr. Stillman	09/01/06	08/31/11	38,421
NIH/Carnegie Mellon University Consortium Agreement	Dr. Osten	09/15/10	07/31/11	134,000 *
NIH/Columbia University Consortium Agreement	Dr. Lowe	08/18/06	07/31/11	402,398
NIH/Cornell University Consortium Agreement	Dr. Mitra	04/03/08	01/31/11	55,118
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	07/31/11	297,852
NIH/Northwestern University Consortium Agreement	Dr. Osten	09/15/09	08/31/11	33,600
NIH/Paratek Pharmaceuticals, Inc. Consortium Agreement	Dr. Krainer	01/05/09	12/31/10	168,931
NIH/Princeton University Consortium Agreement	Dr. Zador	05/01/10	04/30/11	43,500 *
NIH/University of California, San Francisco Consortium Agreement	Dr. Egeblad	09/01/09	08/31/14	178,984
NIH/University of California, Berkeley National Laboratory Consortium Agreement	Dr. Gingeras	05/04/07	03/31/11	655,799
NIH/University of Southern California Consortium Agreement	Dr. Hannon	07/01/10	06/30/14	121,800 *
NIH/University of Southern California Consortium Agreement	Drs. Lowe/Sordella	09/28/09	07/31/14	370,000
NIH/University of Texas, Dallas Consortium Agreement	Drs. Krainer/McCombie/M. Zhang	08/01/10	07/31/11	291,888 *
Fellowship Support	Dr. Borges	08/16/09	08/15/12	52,154
	M. Lazarus	07/15/09	07/14/10	25,380

[1]Includes direct and indirect costs
[2]Award issued under the American Recovery and Reinvestment Act of 2009
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
Graduate Training Support	Dr. Joshua-Tor, Watson School of Biological Sciences	07/01/07	06/30/12	$ 207,432
	Dr. Joshua-Tor, Watson School of Biological Sciences	09/01/09	08/31/11	41,266 [2]
Course Support	Immunocytochemistry, In Situ Hybridization, Super-Resolution, and Live Cell Imaging	09/01/10	08/31/15	78,167 *
	Advanced Techniques in Molecular Neuroscience	07/01/01	06/30/12	74,678
	Caenorhabditis elegans	08/01/98	08/31/11	81,739
	Cell and Developmental Biology of *Xenopus*	04/01/09	03/31/14	60,918
	Computational and Comparative Genomics	06/06/91	07/31/13	50,282
	Eukaryotic Gene Expression	01/01/83	03/31/11	108,929
	Imaging Structure and Function in the Nervous System	07/01/01	06/30/12	87,131
	Integrated Data Analysis for High-Throughput Biology	09/01/07	04/30/12	79,188
	Molecular Embryology of the Mouse	01/01/83	03/31/11	108,369
	Neurobiology of *Drosophila*	07/01/01	06/30/12	68,010
	Programming for Biology	07/07/00	08/31/14	61,800
	Protein Purification and Characterization	01/01/83	03/31/11	86,303
	Proteomics	07/01/03	06/30/11	80,080
	X-Ray Methods in Structural Biology	07/01/07	06/30/12	77,056
	Yeast Genetics and Genomics	09/18/10	06/30/13	51,905 *
Meeting Support	Axon Guidance, Synaptic Plasticity, and Regeneration	08/01/10	07/31/11	10,000 *
	The Biology of Genomes	04/01/08	03/31/13	38,193
	The Cell Cycle	03/01/06	02/28/11	4,000
	Gene Expression and Signaling in the Immune System	04/01/08	03/31/13	10,000
	Molecular Genetics of Aging	03/01/08	02/28/13	30,030
	Germ Cells	07/01/08	06/30/13	6,000
	Glia in Health and Disease	07/01/10	06/30/11	24,989 *
	Mechanisms and Models of Cancer	08/01/08	07/31/13	6,000
	Molecular Chaperones and Heat Shock Response	05/01/10	04/30/13	20,000 *
	Molecular Genetics of Bacteria and Phages	07/01/10	06/30/11	18,500 *
	Mouse Development, Genetics, and Genomics	08/01/10	07/31/11	8,500 *
	Pharmacogenomics and Personalized Therapy	09/30/10	08/31/15	15,000 *
	PTEN Pathways and Targets	03/01/06	02/28/11	7,000
	75th Symposium: Nuclear Organization and Function	04/01/10	03/31/11	10,000 *
	Systems Biology: Global Regulation of Gene Expression	03/01/10	02/28/15	5,000 *
	Telomeres and Telomerase	09/30/10	08/31/11	30,625 *
	Vertebrate Organogenesis	04/01/10	03/31/15	12,000 *

HEALTH RESOURCES AND SERVICES ADMINISTRATION

Equipment Support	Dr. D. Spector	08/01/10	07/31/12	495,000 *

NATIONAL SCIENCE FOUNDATION

Multiple Project Award Support	Dr. Jackson	10/01/10	09/30/15	1,120,694 *

[1]Includes direct and indirect costs
[2]Award issued under the American Recovery and Reinvestment Act of 2009
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
	Drs. McCombie/Martienssen/Ware	08/01/10	07/31/12	$ 2,499,992 *
	Dr. Ware	10/01/07	09/30/11	1,757,922
Research Support	Dr. Jackson	09/01/10	08/31/13	200,000 *
	Dr. Jackson	07/01/09	06/30/11	78,072
	Dr. Lippman	09/15/09	08/31/12	166,841
	Dr. Timmermans	08/15/10	07/31/13	200,000 *
	Dr. Ware	06/15/08	05/31/11	263,886
Research Subcontracts				
NSF/Cornell University Consortium Agreement	Drs. Timmermans/Micklos	09/01/08	08/31/12	408,333
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	08/01/10	07/31/14	384,900 *
NSF/University of Arizona Consortium Agreement	Dr. Ware	08/15/10	07/31/14	542,429 *
NSF/University of Arizona Consortium Agreement	Drs. Stein/Martienssen/Micklos/Ware	02/01/08	01/31/13	2,101,833
NSF/University of California, Berkeley Consortium Agreement	Dr. Jackson	08/01/06	07/31/11	228,512
NSF/University of California, Davis Consortium Agreement	Dr. Ware	09/01/08	08/31/11	214,214
NSF/Washington University Consortium Agreement	Dr. Ware	03/01/10	02/28/11	312,400 *
Fellowship Support	K. Liberatore	06/01/10	05/31/13	40,500 *
Undergraduate Training Support	Dr. Lippman	03/01/09	02/29/12	69,420
Course Support	Advanced Bacterial Genetics	07/01/09	06/30/14	90,953
	Computational Cell Biology	04/15/08	03/31/11	96,338
	Molecular Techniques in Plant Science	07/01/10	06/30/11	96,680 *
Meeting Support	Molecular Genetics of Bacteria and Phages	08/01/10	07/31/11	7,000 *
	7th International Conference on Plasmodesmata	03/01/10	02/28/11	15,000 *
	Systems Biology: Global Regulation of Gene Expression	06/01/10	05/31/11	7,000 *

UNITED STATES DEPARTMENT OF AGRICULTURE

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding
Research Support	Dr. Jackson	09/01/08	08/31/11	133,795
	Dr. McCombie	09/11/09	09/10/14	113,436

UNITED STATES DEPARTMENT OF ENERGY

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding
Research Support	Dr. Martienssen	09/03/10	09/02/12	852,567 *

UNITED STATES DEPARTMENT OF THE ARMY

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding
Research Support	Dr. Hannon	09/01/08	08/31/13	658,118
	Drs. Lucito/Tonks	07/01/10	06/30/12	434,000 *
	Dr. Trotman	08/15/09	08/14/12	162,162

[1]Includes direct and indirect costs
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
	Drs. Wigler/Hicks/Krasnitz	09/01/09	08/31/11	$ 2,151,000
	Dr. Zhong	06/15/10	06/14/13	285,477 *
Fellowship Support	Dr. B. Zhang	03/15/10	03/14/11	61,772 *

MISCELLANEOUS SOURCES OF FUNDING

Equipment Support

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding
F.M. Kirby Foundation	Dr. Egeblad	05/01/10	04/30/11	100,000 *
Eleanor Schwartz Charitable Foundation	Dr. McCombie	06/01/10	05/31/11	200,000 *

Program Project Support

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding
The Leukemia & Lymphoma Society	Dr. Lowe	10/01/08	09/30/13	1,250,000
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lippman/Martienssen/ Timmermans/Ware	07/01/07	06/30/12	1,600,000
The Simons Foundation/Autism	Dr. Wigler	07/01/08	06/30/11	6,664,189
The Simons Foundation/Center for Quantitative Biology	Dr. Wigler	09/01/08	08/31/11	1,500,000
Theodore R. and Vada S. Stanley	Drs. Watson/McCombie	07/01/07	06/30/12	5,000,000

Research Support

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding
Paul G. Allen Family Foundation	Dr. Zador	10/01/10	09/30/13	611,100 *
Alpern Family Foundation Inc.	Dr. Egeblad	12/15/10	12/14/11	2,500 *
Alzheimer's Association	Dr. Furukawa	03/01/10	02/28/12	49,969 *
American Cancer Society	Dr. Wigler	01/01/10	12/31/10	10,000 *
	Dr. Wigler	01/01/06	12/31/10	60,000
Anonymous	Dr. Osten	09/01/09	08/31/11	248,492
The A&P Foundation	Dr. Egeblad	06/01/10	05/31/11	20,000 *
The Breast Cancer Research Foundation	Dr. Wigler	10/01/10	09/30/11	223,000 *
Brookhaven National Laboratory/New York State Department of Health	Dr. Enikolopov	03/01/10	04/30/10	16,202 *
Carnegie Mellon University/Anonymous	Dr. Osten	01/01/10	11/30/11	25,000 *
Mr. and Mrs. Edward Chernoff in honor of Fred and Marika Fogelman	Dr. Egeblad	07/01/10	06/30/11	1,000 *
Mr. and Mrs. Edward Chernoff in memory of James J. Kehoe	Dr. Furukawa	08/15/10	08/14/11	500 *
Clear Channel Worldwide (WALK 97.5 FM)	Dr. Egeblad	04/01/10	03/31/11	4,000 *
Damon Runyon Cancer Research Foundation	Dr. Sordella	01/01/10	12/31/12	150,000 *
The Dana Foundation	Dr. Li	10/01/09	09/30/12	63,844
	Dr. Li	01/01/09	12/31/11	200,000
Dart Neuroscience LLC	Dr. Dubnau	05/01/10	04/30/11	250,000 *
	Dr. Zhong	06/01/10	05/31/11	125,000 *
The Kathryn W. Davis Foundation	Dr. Hannon	01/15/07	01/14/11	1,005,660
Deer Park High School	Dr. Egeblad	12/01/10	11/30/11	500 *
In memory of Raymond Donovan	Dr. Trotman	08/01/10	07/31/11	360 *
The Ellison Medical Foundation	Dr. Enikolopov	09/09/09	09/08/13	252,000
Entertainment Industry Foundation	Dr. Hannon	11/01/10	10/31/11	250,000 *
Charitable Lead Annuity Trust under the Will of Louis Feil	Dr. Osten	12/29/10	12/28/11	25,000 *
Brian Fox through United Way of New York City	Dr. Lowe	03/01/10	02/28/11	2,000 *

[1]Includes direct and indirect costs
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
The Joni Gladowsky Breast Cancer Foundation	Dr. Egeblad	12/01/10	11/30/11	$ 30,000 *
GlaxoSmithKline	Dr. Powers	09/20/06	03/20/11	259,599
Richard and Mindy Gordon	Dr. Egeblad	01/01/10	12/31/10	3,000 *
	Dr. Lazebnik	01/01/10	12/31/10	2,000 *
Greenwich Breast Cancer Alliance Inc.	Dr. Egeblad	01/01/10	12/31/11	62,500 *
The Irving A. Hansen Memorial Foundation	Dr. Tonks	07/01/10	06/30/11	20,000 *
Jo-Ellen and Ira Hazan	Dr. Enikolopov	12/01/10	11/30/11	20,000 *
	Dr. Osten	12/01/10	11/30/11	180,000 *
Hearts for Cancer	Dr. Egeblad	04/01/10	03/31/11	8,455 *
The International Human Frontier Science	Dr. Lippman	07/01/09	06/30/12	100,000
Isis Pharmaceuticals, Inc.	Dr. Krainer	11/01/08	10/31/11	125,000
Islip Breast Cancer Coalition Inc.	Dr. Egeblad	05/01/10	04/30/11	5,000 *
Knott Family Foundation	Drs. Tonks/Pappin	09/01/10	08/31/11	15,000 *
The Susan G. Komen Breast Cancer Foundation, Inc.	Dr. Egeblad	04/13/10	04/12/13	150,000 *
Charles Henry Leach II Foundation	Dr. Enikolopov	06/01/10	05/31/11	2,000 *
Mara and Thomas Lehrman Charitable Fund	Dr. Erlich	08/01/10	07/31/11	40,000 *
The George Link, Jr. Foundation, Inc.	Dr. Sordella	12/01/10	11/30/11	50,000 *
The Long Island 2-Day Walk to Fight Breast Cancer	Dr. Egeblad	08/15/10	08/14/11	31,000 *
The Lustgarten Foundation	Drs. Hannon/Lowe	01/01/10	12/31/12	1,048,204 *
	Drs. Powers/Lowe/Van Aelst	02/01/10	01/31/12	424,723 *
Carol Marcincuk Fund	Dr. Tonks	01/01/10	12/31/10	3,872 *
In honor of Carissa Maringo	Dr. Egeblad	10/01/10	09/30/11	4,540 *
The G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Mitra	09/01/10	08/31/13	204,135 *
Breast Cancer Awareness Day in memory of Elizabeth McFarland	Dr. Wigler	01/01/10	12/31/11	72,305 *
The McKnight Endowment Fund for Neuroscience	Dr. Osten	08/01/09	07/31/11	100,000
The John Merck Fund	Dr. Kepecs	06/01/10	05/31/14	75,000 *
The Don Monti Memorial Research Foundation	Dr. Lowe	03/01/10	02/28/11	300,000 *
Louis Morin Charitable Trust	Dr. Joshua-Tor	12/01/10	11/30/11	70,000 *
The Neuberger Berman Foundation	Dr. Egeblad	02/01/10	01/31/11	20,000 *
New York State Department of Health– New York Stem Cell Science (NYSTEM)	Drs. Enikolopov/Koulakov	01/01/09	12/31/11	360,000
	Dr. Timmermans	01/01/09	12/31/10	119,900
Paratek Pharmaceuticals, Inc.	Dr. Krainer	01/01/10	12/31/10	37,800 *
Philips Research North America	Drs. Lucito/Wigler	04/01/09	03/31/11	636,518
	Dr. Zhang	01/01/09	12/31/10	227,527
V. Kann Rasmussen Foundation	Dr. Trotman	09/01/07	08/31/12	50,000
Christina Renna Foundation, Inc.	Dr. Van Aelst	01/01/08	12/31/11	15,000
Diane Emdin Sachs Memorial Fund	Dr. Sordella	09/01/10	08/31/11	13,315 *
Judi Shesh Memorial Foundation	Dr. Egeblad	08/01/10	07/31/11	10,000 *
The Simons Foundation	Dr. Henn	09/01/10	08/31/12	294,030 *
	Dr. Henn	09/01/10	08/31/11	500,978 *
	Dr. Mills	01/01/10	10/31/10	289,633 *
	Dr. Mills	07/01/10	06/30/13	350,000 *
	Dr. Mitra	09/01/09	08/31/11	150,000
	Dr. Osten	07/01/09	06/30/11	150,107

[1]Includes direct and indirect costs
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
Mary F. Smith Family Foundation	Dr. Egeblad	11/01/10	10/31/11	$ 5,000 *
Mr. and Mrs. Douglas S. Soref	Drs. Tonks/Pappin	09/01/10	08/31/11	15,000 *
Starr Cancer Consortium	Dr. Egeblad	08/01/09	07/31/11	150,000
	Dr. Krainer	11/01/10	10/31/11	240,000 *
	Dr. Lazebnik	08/01/09	07/31/11	180,000
	Drs. Lowe/Vakoc	08/01/10	07/31/12	167,500 *
	Drs. Lucito/Tonks	08/01/09	07/31/11	491,076
	Dr. McCombie	08/01/09	07/31/11	180,000
	Dr. Powers	08/01/09	07/31/11	180,000
	Drs. Sordella/Van Aelst	08/01/10	07/31/12	809,381 *
	Dr. Trotman	08/01/09	07/31/11	150,000
	Dr. Wigler	08/01/09	07/31/11	854,942
	Drs. M. Zhang/Atwal	08/01/09	07/31/11	240,000
The Sandra and Howard Tytel Family Foundation, Inc.	Drs. Tonks/Pappin	09/01/10	08/31/11	15,000 *
University of California, Los Angeles/ Entertainment Industry Foundation	Dr. Hannon	10/01/09	09/30/12	828,568
U.S.–Israel Binational Agricultural Research and Development Fund	Dr. Lippman	06/01/10	05/31/13	41,000 *
	Dr. Krainer	10/01/10	09/30/14	10,000 *
	Dr. Zador	10/01/10	09/30/14	5,750 *
The V Foundation	Drs. Powers/Sordella	11/01/08	10/31/11	200,000
West Islip Breast Cancer Coalition for Long Island Inc.	Dr. Egeblad	11/01/10	10/31/11	10,000 *
Whitehall Foundation, Inc.	Dr. Kepecs	06/01/08	05/31/11	75,000
Ms. Evelyn Willard	Dr. Churchland	12/21/10	12/20/11	50,000 *
Women's Partnership Lectures	Dr. Sordella	12/01/10	11/30/11	1,350 *

Fellowship Support

Australian Government National Health and Medical Research Council	Dr. Bolden	04/01/10	03/31/12	76,230 *
	Dr. Dow	02/01/09	01/31/11	45,478
Terri Brodeur Breast Cancer Foundation	Dr. Bialucha	01/01/09	12/31/10	50,000
Canadian Institutes of Health Research	Dr. Aznarez	07/01/09	12/31/11	43,044
Cashin Family Fund	Watson School of Biological Sciences	09/01/09	08/31/11	80,000
The Jane Coffin Childs Memorial Fund for Medical Research	Dr. Kuhn	03/29/10	01/31/12	39,475 *
CSHL Association Fellowship	New Investigator Support	01/01/10	12/31/10	280,000 *
Deutsche Forschungsgesellschaft	Dr. Ahrens	06/01/10	05/31/12	43,296 *
Engelhorn Scholarship Program	Watson School of Biological Sciences	01/01/10	12/31/10	49,980 *
Genentech Foundation	Watson School of Biological Sciences	07/01/09	06/30/12	57,157
The Fred C. Gloeckner Foundation, Inc.	J. Calarco	08/01/10	07/31/11	8,100 *
The Allen and Lola Goldring Foundation	Dr. Stillman	09/01/10	08/31/11	75,000 *
Lita Annenberg Hazen Foundation	Watson School of Biological Sciences	05/01/08	04/30/18	10,000
Hope Funds for Cancer Research	Dr. Sawey	07/01/09	06/30/11	44,500
Human Frontier Science Program	Dr. Canela	05/01/09	04/30/11	53,640
	Dr. Kvitsiani	09/01/08	08/31/11	47,150
	Dr. Skopelitis	04/01/09	03/31/12	51,720
International Rett Syndrome Foundation	Dr. Krishnan	10/01/10	09/30/12	50,000 *
	Dr. Lin	01/01/09	12/31/10	50,000
	Dr. Paul	10/01/09	09/30/11	50,000
Annette Kade Charitable Trust	Watson School of Biological Sciences	04/01/10	03/31/11	50,000 *
Harvey L Karp Foundation	Dr. Ipsaro	09/01/10	08/31/11	50,000 *
The Esther A. & Joseph Klingenstein Fund Inc.	Dr. Kepecs	07/01/09	06/30/12	50,000
	Dr. Shea	07/01/10	06/30/13	50,000 *

[1]Includes direct and indirect costs
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
The Susan G. Komen Breast Cancer Foundation, Inc.	Dr. Anczukow	06/04/09	06/03/12	$ 60,000
Life Sciences Research Foundation	Dr. Xu	11/01/09	10/31/12	56,000
NARSAD	Dr. Karakas	01/01/10	12/31/11	30,000 *
	Dr. Taniguchi	01/01/10	12/31/11	30,000 *
National Cancer Center	Dr. Mao	07/01/10	06/30/12	40,000 *
The Robert Leet and Clara Guthrie Patterson Trust	Dr. Lu	01/15/09	01/14/11	46,000
Mr. and Mrs. John C. Phelan	Watson School of Biological Sciences	09/01/10	08/31/11	100,000 *
Marie Robertson Memorial Fund	New Investigator Support	01/01/10	12/31/11	137,500 *
The Seligson Foundation	Dr. Zuber	01/01/10	12/31/10	75,000 *
Alfred P. Sloan Foundation	Dr. Kepecs	09/16/09	09/15/11	25,000
Lauri Strauss Leukemia Foundation	Dr. Chien	06/01/10	05/31/11	10,000 *
The Swartz Foundation	Dr. Zador	01/01/10	12/31/10	100,000 *
	Drs. Koulakov/Kolterman	01/01/10	12/31/10	55,000 *
	Drs. Zador/Reid	01/01/10	12/31/10	55,000 *
	Dr. Zador	01/01/10	12/31/10	7,500 *

Training Support

Howard Hughes Medical Institute	Undergraduate Research Program	06/01/10	05/31/11	4,500 *
Research Foundation of the City University of New York	Undergraduate Research Program	06/01/10	05/31/11	7,000 *
Steamboat Foundation	Undergraduate Research Program	05/01/10	04/30/11	12,000 *
William Townsend Porter Foundation	Undergraduate Research Program	04/01/10	03/31/11	10,010 *

Course Support

American Brain Tumor Association	Brain Tumors	01/01/10	12/31/10	45,000 *
American Express Foundation	Leadership in Bioscience Workshop	01/01/10	12/31/10	95,000 *
Applied Biosystems	Advanced Sequencing Technologies and Applications	07/01/10	06/30/11	30,000 *
Autism Speaks, Inc.	Workshop on Biology of Social Cognition	05/01/07	04/30/11	15,000
Howard Hughes Medical Institute	Neurobiology Course Support	01/01/07	12/31/11	750,000
Illumina, Inc.	Advanced Sequencing Technologies and Applications	07/01/10	06/30/11	30,000 *
The Nancy Lurie Marks Family Foundation	Workshop on Biology of Social Cognition	05/01/07	04/30/11	15,000
Roche Diagnostics Corporation	Advanced Sequencing Technologies and Applications	07/01/10	06/30/11	30,000 *
	Workshop on Circuit and Molecular Architecture	07/01/10	06/30/11	40,000 *
The Simons Foundation	Workshop on Biology of Social Cognition	05/01/07	04/30/11	15,000

Meeting Support

Alnylam US, Inc.	RNA and Oligonucleotide Therapeutics	04/01/10	03/31/11	1,000 *
Amgen Inc.	Gene Expression and Signaling in the Immune System	10/01/09	09/30/10	5,000
AVI BioPharma, Inc.	RNA and Oligonucleotide Therapeutics	04/01/10	03/31/11	25,000 *
Blake Industries	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000 *
Boehringer Ingelheim Pharmaceuticals Inc.	Gene Expression and Signaling in the Immune System	10/01/09	09/30/10	2,500
Cambridge Isotope Laboratories, Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000 *
Genentech, Inc.	Vertebrate Organogenesis	09/01/09	08/31/10	3,500

[1]Includes direct and indirect costs
*New grants awarded in 2010

Grantor	Program/Principal Investigator	Duration of Grant		2010 Funding[1]
Genentech, Inc.	Gene Expression and Signaling in the Immune System	10/01/09	09/30/10	$ 2,500
Helixis, Inc.	The Biology of Genomes	04/01/10	03/31/11	7,500 *
Illumina, Inc.	Personal Genomes	09/14/09	09/13/10	3,750
	The Biology of Genomes	04/01/10	03/31/11	11,000 *
Isis Pharmaceuticals, Inc.	RNA and Oligonucleotide Therapeutics	04/01/10	03/31/11	3,000 *
The March of Dimes Foundation	Vertebrate Organogenesis	03/01/10	08/31/10	5,000 *
Merck Serono S.A.	Gene Expression and Signaling in the Immune System	10/01/09	09/30/10	10,000
Molecular Devices, Inc.	Automated Imaging and High-Throughput Phenotyping	11/01/10	10/31/11	5,000 *
New England BioLabs, Inc.	Infectious Disease Genomics and Global Health	08/01/10	07/31/11	1,500 *
Openeye Scientific Software, Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	2,000 *
Penn State University	Galaxy Developers	04/01/10	03/31/11	35,050 *
Pfizer Inc.	RNA and Oligonucleotide Therapeutics	04/01/10	03/31/11	3,000 *
Roche Diagnostics Corporation	Personal Genomes	03/01/10	02/28/11	49,466 *
Schrodinger	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	1,000 *
Shigemi Inc.	Protein Data Bank 40th Anniversary	12/01/10	11/30/11	500 *
Alfred P. Sloan Foundation	New Faculty Investigator Conference	12/01/10	06/01/11	20,000 *

Library Support

Grantor		Duration of Grant		2010 Funding
Ellen Brenner Memorial Fund		12/22/10	12/21/11	2,000 *
New York University School of Medicine		01/26/10	04/30/10	1,000 *
The State of New York		07/01/09	06/30/10	3,850

[1]Includes direct and indirect costs
*New grants awarded in 2010

DNA LEARNING CENTER GRANTS

Grantor	Program	Duration of Grant	2010 Funding[†]
FEDERAL GRANTS			
National Institutes of Health	Science Education Partnership Award (SEPA): Nationwide Dissemination of *Inside Cancer* Internet Site	8/08–7/11	$ 52,701
National Science Foundation	Course, Curriculum, and Laboratory Instruction (CCLI) Program: Nationwide Dissemination of RNAi Curriculum	9/07–8/11	87,245
National Science Foundation, Cornell University	*Weed to Wonder* Internet Site Development: Educational Outreach for Functional Genomics of the Maize Shoot Apical Meristem	9/08–8/11	74,732
National Science Foundation, University of Arizona	Educational Outreach for *iPlant*: A Cyberinfrastructure for Plant Sciences	2/08–1/13	720,501
NONFEDERAL GRANTS			
Bank of America	Scholarships for Minority and Underserved Students on Long Island	2010	25,000
Dana Foundation	*Harlem DNA Lab* Operating Support	3/09–2/12	129,334
Hewlett Foundation	*Genes to Cognition Online* Internet Site Dissemination and Evaluation	10/08–4/11	37,190
Howard Hughes Medical Institute	Pre-College Science Education Initiative: NYC Teacher Professional Development	9/07–8/12	142,476
Lounsbery Foundation	Biotechnology Footlocker Program at *Harlem DNA Lab*	11/09–10/10	21,546
National Grid Foundation	Scholarships for Minority and Underserved Students in the Brentwood Union Free School District	10/10–10/11	15,000
North Shore–LIJ Health System	DNALC *West* Operating Support	2010	50,000
William Townsend Porter Foundation	Scholarships for Minority and Underserved Students at *Harlem DNA Lab*	3/10–3/11	35,035

The following schools and school districts each contributed $1000 or more for participation in the *Curriculum Study* Program:

Bellmore–Merrick Central High School District	$ 3,000	Northport–East Northport Union Free School District	$ 2,000
Bethpage Union Free School District	1,500	Oyster Bay–East Norwich School District	1,500
East Meadow Union Free School District	1,500	Plainview–Old Bethpage Central School District	1,500
Great Neck Union Free School District	1,500	Portledge School	1,500
Harborfields Central School District	1,500	Roslyn Union Free School District	1,500
Herricks Union Free School District	1,500	Syosset Central School District	1,500
Long Beach City School District	1,500	The Green Vale School	1,500
Massapequa Union Free School District	1,500	Yeshiva University High School for Girls	1,500

The following schools and school districts each contributed $1000 or more for participation in the *Genetics as a Model for Whole Learning* Program:

Bay Shore Union Free School District	$ 6,000	Locust Valley Central School District	$ 9,989
Bellmore Union Free School District	3,500	Merrick Union Free School District	2,250
Bellmore–Merrick Central HS District	8,000	MS 447–The Math and Science Exploratory School, NYC	3,000
Commack Union Free School District	6,700	North Bellmore Union Free School District	1,600
Copiague Union Free School District	1,200	Northport–East Northport Union Free School District	1,025
Eastern Middle School, Greenwich, CT	3,000	North Shore Central School District	1,500
East Meadow Union Free School District	5,550	Oceanside Union Free School District	1,500
East Williston Union Free School District	2,800	Oyster Bay–East Norwich Central School District	3,050
Elwood Union Free School District	4,500	Palisades Middle School, PA	1,800
Floral Park–Bellerose Union Free School District	6,250	Rockville Centre Union Free School District	2,400
Friends Academy, Locust Valley	2,800	Roslyn Union Free School District	3,599
Garden City Union Free School District	11,500	Scarsdale Union Free School District	10,500
Great Neck Union Free School District	16,550	Smithtown Central School District	1,400
Half Hollow Hills Central School District	7,625	Saint Dominic Elementary School, Oyster Bay	4,050
Herricks Union Free School District	3,150	St. Edward the Confessor School, Syosset	2,025
Holy Child Academy, Westbury	6,500	Syosset Central School District	32,500
Huntington Union Free School District	6,700	Three Village Central School District	1,500
Island Park Union Free School District	1,350	Valley Stream 13 Union Free School District	2,400
Jericho Union Free School District	7,875	Yeshiva Darchei Torah School	1,600
Kings Park Central School District	1,950	Yeshiva of Flatbush	1,800
Lawrence Union Free School District	7,100		

[†]Includes direct and indirect costs

BANBURY CENTER GRANTS

Grantor	Program	Duration of Grant	2010 Funding
FEDERAL SUPPORT			
NIH–National Institute of Mental Health	The Second NIMH-Sponsored Brain Camp	2010	$ 44,465
The U.S. Department of Homeland Security	Microbial Forensics in the Era of Genomics	2010	23,433
NONFEDERAL SUPPORT			
Abraxis Bioscience, Inc.	The Calculus of Medicine: Treatment of Pancreatic Cancer as a Prime Exemplar	2010	41,955
Alfred P. Sloan Foundation	How Can We Maintain the Stability of Biomedical Research and Development at the End of the ARRA?	2010	9,891
The ALS Association	Stem Cells, Genetics, and RNA-Binding Proteins: Recent Advances in ALS Research and Drug Discovery	2010	32,231
Champalimaud Foundation and the Champalimaud Metastasis Programme	Tumor Microenvironment and Metastasis	2010	35,407
Cold Spring Harbor Laboratory Corporate Sponsor Program	Epigenetic Reprogramming and Transgenerational Inheritance	2010	52,673
Cold Spring Harbor Laboratory Corporate Sponsor Program	Signaling through Ubiquitin	2010	45,994
Cold Spring Harbor–Pioneer Collaborative Research Program	Plant Development and Phenomics	2010	54,394
Columbia University	p53 Retreat	2010	10,870
The Ellison Medical Foundation	Easeful Death: 21st Century Perspectives on Assisted Suicide	2010	25,374
Howard Hughes Medical Institute	How Can We Maintain the Stability of Biomedical Research and Development at the End of the ARRA?	2010	10,000
The Lehrman Institute	DNA, Genetics, and the History of Mankind	2010	29,755
Marie Robertson Research Fund	The Lateral Habenula: Its Role in Behavior and Psychiatric Disorders	2010	40,000
Oliver Grace Fund	Linguistic Phenotypes: Toward a Biological Understanding of Language	2010	31,472
Oliver Grace Cancer Fund	Mutagenesis: What It Means and How It Has Changed	2010	39,357
Oliver Grace Cancer Fund	Energy Metabolism, the Cell Cycle, and Cancer	2010	40,912
PopTech Accelerator	PopTech Science and Public Leadership Fellows Retreat	2010	42,000
The Simons Foundation	Linguistic Phenotypes: Toward a Biological Understanding of Language	2010	10,000
United Biomedical, Inc.	Genetic Variation at a Single Locus for Prediction and Prevention of Late-Onset Alzheimer's Disease	2010	14,275
Université de Québec	Linguistic Phenotypes: Toward a Biological Understanding of Language	2010	3,000
University of Illinois	Fragile X Syndrome: Current Status, Future Prospects	2010	48,306
Various institutions and individuals	Genome-Era Pathology, Precision Diagnostics, and Preemptive Care: A Stakeholder's Summit	2010	22,260

CORPORATE SPONSOR PROGRAM
FOR MEETINGS SUPPORT

The Corporate Sponsor Program continues to provide critical funding for the vigorous meetings program held at Cold Spring Harbor Laboratory, whether at Grace Auditorium on the main Laboratory campus or at the Banbury Center. Without the strong foundation provided by the Program, we could neither plan with confidence for the year's meetings nor introduce new and unusual topics.

We are especially grateful to the companies that joined us in 2010 as the economic difficulties began to take effect. The year 2011 is going to be especially challenging as the number of companies shrinks through takeovers, and companies and foundations adopt austerity measures.

The members of the Program receive special privileges in acknowledgment of their contributions. We waive all on-site fees for eight representatives of the Corporate Sponsors at our meetings. Three scientists from Sponsors may attend meetings at the Banbury Center, where attendance is otherwise only by invitation of the organizers. Member companies also receive gratis copies of Cold Spring Harbor Laboratory Press publications, including the journals *Genes & Development, Learning & Memory, Protein Science, Genome Research*, and *RNA*.

We acknowledge our Sponsors in all relevant publications, including the books of abstracts given to each of the 7000 participants who come to the meetings each year. The names of the sponsoring companies are listed on the poster describing the meetings, and this is mailed to approximately 17,000 scientists throughout the world. In addition, the companies are listed on the Cold Spring Harbor Laboratory website on the Meetings Office and Banbury Center pages. Members in 2010 were the following:

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DEVELOPMENT

The completion of the $200 million campaign in 2009 brought many new fund-raising challenges and opportunities. Hiring of faculty to fill the new space became a top priority as did the need for additional resources for recruiting, staffing, and equipping the buildings. We once again turned to our Board of Directors, closest friends, and local community groups to ensure that the Laboratory continued its tradition of bringing the best and brightest young minds here.

In 2010, the Laboratory lost two very good friends, Charlie Harris and George "Butch" Cutting. Charlie was a former Trustee and an early member and Co-Chair of our President's Council. Ironically, his passion for nanotechnology was the inspiration for the 2010 President's Council meeting. Butch was a former Trustee, Honorary CSHL Association member, and longtime supporter. It was never a surprise to see him at the Laboratory's many dinners, concerts, lectures, and fund-raising events. Both Charlie and Butch were great advocates and staunch supporters of the Laboratory during many years and they will be missed deeply.

The Double Helix Medals Dinner continues to be a source of pride for the Laboratory as we recognize some of the most influential scientists, community leaders, and business executives in the biomedical field. This year's event honored Evelyn Lauder, John Nash, and Mary-Claire King for their enormous achievements. It also raised more than $3 million in important unrestricted funding for the Laboratory.

On a personal note, I would like to thank Eduardo Mestre for his 6 years of dedicated service as Chairman of the Board. Under his leadership, the Laboratory has grown in every way, and most importantly, our researchers continue to produce some of the world's most outstanding science. There is no doubt that Jamie Nicholls will take over right where Eduardo left off in leading the Laboratory's efforts in science and discovery. I look forward to working closely with her in this endeavor.

2010 Double Helix Medals honorees John Nash, Mary-Claire King, and Evelyn Lauder

We at the Laboratory remain eternally grateful for the support we receive from our community, advocates, supporters, and friends. Thank you for helping us to strengthen and expand our science and educational programs not only on Long Island, but all across the United States and the world.

Charles V. Prizzi, *Vice President for Development*

Cold Spring Harbor Laboratory is a nonprofit research and educational institution chartered by the State of New York. Less than half of the Laboratory's annual revenues are derived from federal grants and contracts, and thus, it relies heavily upon support from the private sector: foundations, corporations, and individuals. CSHL takes this occasion to sincerely thank each and every donor whose generous contributions have made possible the discoveries we report in this publication. There are a variety of ways to give to the Laboratory:

Capital and Endowment Campaign Support: Donations help secure the financial stability of the Laboratory, increase the speed that genetic discoveries are translated into diagnostic tests and therapeutics, and expand the Lab's facilities and staff.

Science Education: Donations support programs at the DNA Learning Center and the Watson School of Biological Sciences, where the next generation of citizens learns about genetics in an exciting and interactive environment.

Annual Fund: Donations provide funding for some of the most determined and innovative young researchers in science today and constitute an important investment in innovative research in cancer, neuroscience, plant biology, and bioinformatics.

Planned and Estate Gifts: Individuals who inform us of their intention to make a gift to CSHL from their estate are invited to become members of The Harbor Society. Estate gifts help to ensure that CSHL will continue to pursue its mission for many years to come.

For additional information, please contact Charlie Prizzi, Vice President for Development, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6961.

President's Council

The 2010 President's Council topic, "Tiny Treatments: The Science of Nanotechnology," featured talks on nanotech investment, potential treatments for brain trauma, new approaches for diagnostics and drug delivery, and safety concerns. Members enjoyed the April reception in New York hosted by long-time Lab friends Nathalie and Bill Comfort in their spacious penthouse with breathtaking views. Co-chairs of the council this year were Thomas Lehrman, Cynthia Stebbins, and Jim Stone. The October meeting was held for the first time on our new Hillside Campus and included talks by nanotech luminaries Bob Langer and George Whitesides.

The increasingly timely topic of nanotechnology was chosen in part as a tribute to Charles E. Harris, the late CSHL Trustee and founding member and former Chairman of the President's Council. Charlie, founder of Harris & Harris Group, a publicly traded nanotech venture firm, was among the Lab's most beloved friends. After learning of his incurable cancer in March 2009, Charlie began to write a blog about dying—and living—written with the warmth, eloquence, and insight so characteristic of him. Charlie, who worked behind the scenes to shape this year's particularly dynamic meeting, died the day before it took place. We look forward to the release of *Incurable: A Life after Diagnosis*, a publication of his blog to be released this spring by CSHL Press.

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Isaac Cheng, Howard Morgan, Charles Prizzi, and Ginny Knott at the President's Council October meeting.

Susan Harris and Nathalie Comfort at the Spring President's Council reception.

Cold Spring Harbor Laboratory Association (CSHLA)

With nearly 1000 members, the CSHLA and its directors continue to increase awareness of CSHL. This year, nearly $5,000,000 in unrestricted support was raised for the Laboratory. Events and benefits spearheaded by directors of the Association and members of the Board of Trustees included the Double Helix Medals dinner in New York City, Symposium dinner parties, a regional junior chess tournament, dinners and luncheons highlighting the accomplishments of our female scientists, and a variety of receptions and dinners to introduce new friends to the exceptionally talented young scientists who devote their lives to improving human health.

Early in 2010, we lost George "Butch" Cutting. Butch was an enthusiastic champion of the Laboratory for many decades as Association director, president, honorary director, and member of the Board of Trustees and the Laboratory's President's Council. He helped to shape the Laboratory Association board of directors in its early days and put the "fun" into fund-raising for scientific research. He was genuinely interested in the research process and enjoyed numerous enduring friendships with scientists, a great asset in his quest to help demystify molecular biology to friends from Cold Spring Harbor to Alaska where he was always just a phone call away from the Lab's development department each summer. We are grateful to him for his true interest and for continuing with gusto the tradition of neighborly support that is unique to Cold Spring Harbor Laboratory.

Special thanks go to Tim Broadbent for his thoughtful leadership as Association President for the last 2 years and for his and his wife Lisa's success for the past 7 years raising friends and funds at numerous special events with creativity and elegance.

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CSHLA Members Sandy Pearson (*left*) with Robin and Paul Vermylen

Ashley O'Keefe (*left*) with CSHLA Directors Suzanne DiMaio and Michele Celestino

DNA Learning Center Advisory Boards

We are lucky to have high-level support from two advisory bodies: the Education Committee and the Corporate Advisory Board (CAB). The Education Committee formulates policy and assists with strategic planning, whereas the CAB provides liaison to the Long Island and New York City business community. The CAB also conducts an annual golf tournament and the annual fund campaign. These are the DNALC's major sources of unrestricted funding. In 2010, more than $200,000 was raised through these events.

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CAB member Pat Petersen (*second from left*) with Daniel Gale Sotheby International Realty colleagues during the DNALC Annual Golf Tournament at the Piping Rock Country Club

CAB member Dan Decker (*standing, middle*) and his colleagues from Eppendorf North America at the 2010 Golf Tournament

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CSHL Trustee Jim Stone with Terry Lindsay (*left*) and Cynthia Stebbins at the President's Council Spring Reception held at the home of Nathalie and Bill Comfort

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CSHL Chairman-Elect Jamie Nicholls (*center*) with (from *left to right*) Dan Loeb, Fran Biondi, Hartley Rogers, Amy Falls, and Judy Cormier at the Double Helix Medals Dinner held on November 10, 2010 at the Mandarin Oriental in New York

CSHLA Director Fife Whitman (*left*), Heather Whitman, CSHLA Director Heather Spehr, and Rich Spehr

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CSHL Trustees John Phelan (left) and Roy Zuckerberg with Melva Bucksbaum and Amy Phelan at the Double Helix Medals Dinner

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On December 20, 2010, The Manhasset Women's Coalition Against Breast Cancer presented Dr. Mikala Egeblad (*third from left, front row*) with a check for $50,000 to support her breast cancer research.

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