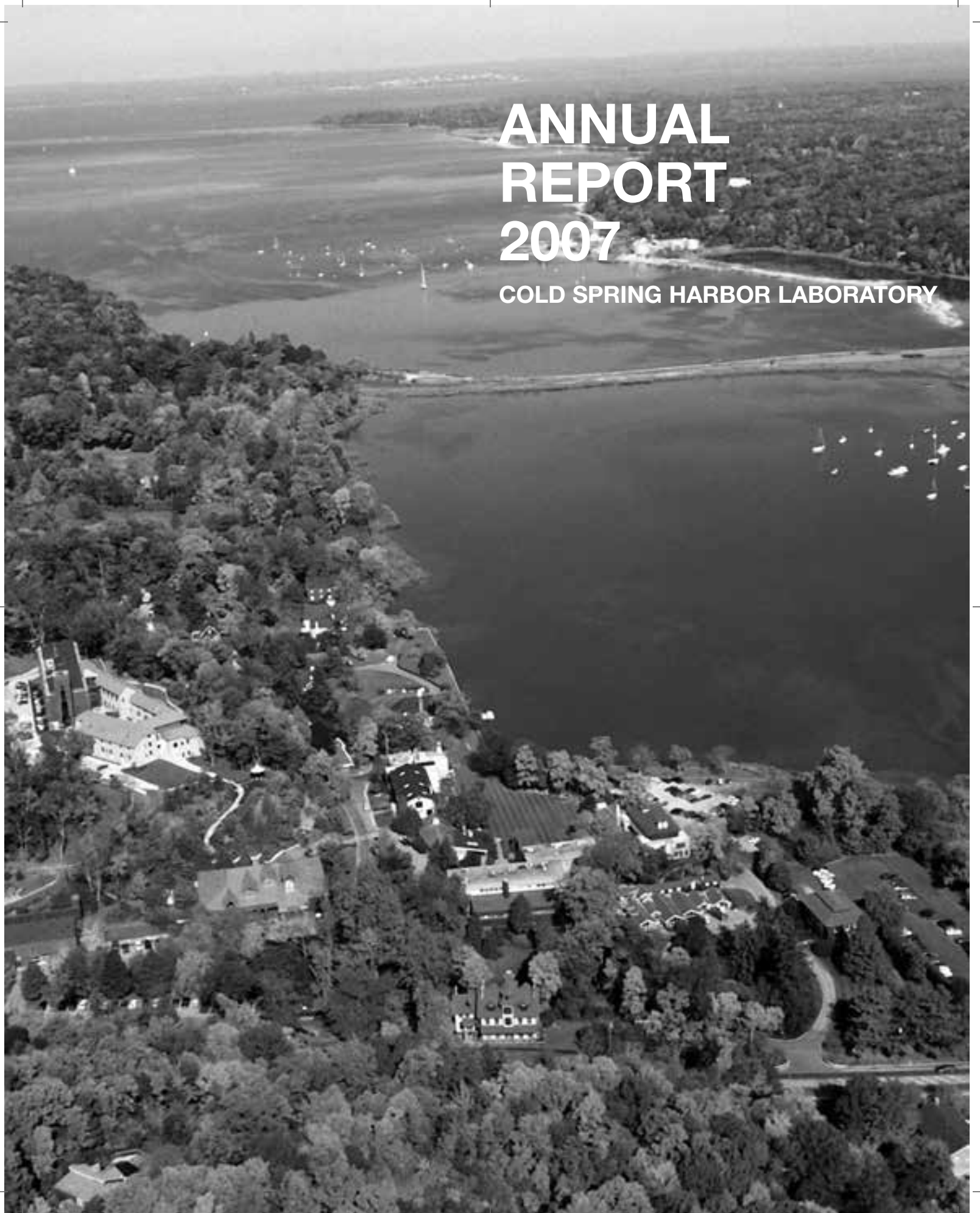

ANNUAL REPORT 2007

COLD SPRING HARBOR LABORATORY

ANNUAL REPORT 2007

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

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Front cover: Newborn cells generated form neural stem and progenitor cells migrate, surrounded by astroglial processes, toward the olfactory bulb where they will become functional neurons. This image, taken at the CSHL Microscopy Shared Resource at a magnification of 1000x, is courtesy of Juan Manuel Encinas, Ph.D.

Section title page photos: Constance Brukin

Contents

Officers of the Corporation and Board of Trustees iv–v
Governance vi
Committees of the Board vii
Martha Farish Gerry (1918–2007) viii
William Edward Murray, Esq. (1926–2007) x

PRESIDENT'S REPORT **1**
Highlights of the Year 3

CHIEF OPERATING OFFICER'S REPORT **22**
Long-term Service 25

CHANCELLOR'S REPORT **26**

RESEARCH **31**
Cancer: Gene Expression and Proliferation 33
Cancer: Genetics 58
Cancer: Signal Transduction 84
Neuroscience 103
Plant Genetics 153
Bioinformatics and Genomics 172
CSHL Fellows 188
Author Index 192

WATSON SCHOOL OF BIOLOGICAL SCIENCES **195**
Dean's Report 197
Courses 211
Undergraduate Research Program 220
Partners for the Future 223

COLD SPRING HARBOR LABORATORY MEETINGS AND COURSES **225**
Academic Affairs 226
Symposium on Quantitative Biology 228
Meetings 232
Postgraduate Courses 280
Seminars 335

BANBURY CENTER **337**
Executive Director's Report 339
Meetings 342

DOLAN DNA LEARNING CENTER **391**
Executive Director's Report 393
2007 Workshops, Meetings, and Collaborations 409

COLD SPRING HARBOR LABORATORY PRESS **415**
2007 Publications 416
Executive Director's Report 417

FINANCE **421**
Financial Statements 422
Financial Support of the Laboratory 425
Grants 425
Development 435
Honor Roll of Donors 439
Corporate Sponsor Program for Meetings Support 445

LABORATORY STAFF **446**

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Martha Farish Gerry (1918–2007)

Martha Farish Gerry counted among her greatest accomplishments her "four children, a happy marriage, and Forego," her three-time "Horse of the Year." Martha is remembered by many as the owner and operator of the Lazy F Ranch, one of the first woman members of the Jockey Club, and the woman who did the most for horse racing (New York Turf Writers, 1974). At Cold Spring Harbor Laboratory, we fondly remember her enthusiasm for biomedical research and education and her generosity that helped to keep those interests alive. Her eyes were always bright with curiosity and wonder. We miss her visits to our laboratories, her enthusiasm for our graduate school, her insightful and direct comments at our board meetings, and her attendance at our scientific lectures. Most of all, we miss her as a very enthusiastic friend who helped to guide this institution over many years.

Our friendship with Martha began in 1989 when she first met my predecessor, Jim Watson, who at the time was Director of CSHL. Martha was modest about her knowledge of science and science education, but her interest grew tremendously over her years of involvement here. Even though history was her major at Vassar from 1936 to 1938, she became a knowledgeable advocate for science during the later years of her life. In addition to what she so enthusiastically absorbed from participating in educational events at CSHL, I understand from her family that she always did her homework in preparation for those events. I wasn't surprised to learn that she made sure to read up on the latest scientific research so that she could keep up in conversations that she would regularly have with Jim and other researchers on campus. Scientists are lifelong learners, and in this aspect of science, Martha was not going to let her lack of formal training hold her back.

Martha Farish Gerry was born to William Stamps Farish II and Libbie Rice Farish in Houston, Texas, on October 20, 1918. Her father was the cofounder of Humble Oil and Refining (later Exxon Company, U.S.A.) and the founder of the American Petroleum Institute. He moved his family to New York when he was named Chairman of Jersey Standard. He was named President 4 years later. Martha's career in horse racing began unexpectedly when her father died of a heart attack at age 61, shortly after retiring and getting interested in horse racing. At the age of 24, Martha took on the responsibility of four horses that her father had recently purchased. You could say the rest is history. Martha owned and operated the Lazy F Ranch, which was originally owned by her father for over 60 years.

In addition to running the ranch and gracing the winner's circle in 34 races with her most famous horse Forego, Martha was recognized throughout the horse-racing world with many honors. Martha was Chairwoman Emeritus of the Board of Trustees of the National Museum of Racing and Hall of Fame in Saratoga, New York. Shortly before her unexpected death following heart surgery on September 17, she was the first woman to be honored as an Exemplar of Racing by the Hall of Fame for her contributions to the sport.

Martha helped to establish the Forego Fund for the Thoroughbred Retirement Foundation. Martha's charitable work did not stop there; the William Stamps Farish Fund broadly supports medical research, social service needs, arts, and education. Martha herself was particularly involved with the Family & Children's Association, New York Presbyterian Hospital, and the United Hospital Fund.

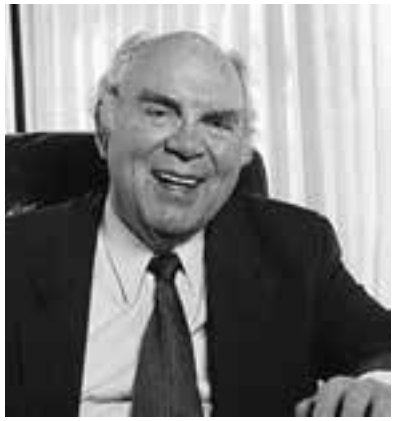
In the CSHL community, Martha participated actively in the governance of the Laboratory. She was a member of the CSHL Board of Trustees from 1991 until her death, serving as Secretary of the Board from 1997 to 2000. She was distinguished by her election as an Honorary Trustee in 2000. Board committees that greatly benefited from her guidance included those overseeing the Banbury Program, the Dolan DNA Learning Center, Buildings, and Development. She delighted, as did we, when the 2004 President's Council meeting brought together Martha's love for science and horse racing by discussing the genetics of thoroughbreds. This meeting highlighted the fascinating history of the thoroughbred horse, the problems incurred by its limited genetic outbreeding, and the precarious physical condition of today's horses that limits increases in performance while causing susceptibility to injury. It was at this meeting that I really saw Martha in her element, for she was more knowledgeable than the "experts," she was keen to see DNA biology have a role in the sport, and she projected a dominant presence and spirit that all who attended noticed and admired.

Martha Farish married Edward H. Gerry (1914–2003), William Averell Harriman's nephew, in 1939. They had four children: William Gerry, Cornelia Corbett, Martha Townley, and Libbie Gerry. Both Martha and Edward and the entire Farish family were generous supporters of many CSHL initiatives ranging from cancer research projects to educational initiatives and campus infrastructure through the William Stamps Farish Fund. Martha believed in CSHL's commitment to discovery and innovation. Thanks to her belief in the fundamental mission of CSHL, we have the Farish Gerry Fellowship for a graduate student, the Edward and Martha Gerry Fellowship for a graduate student, the Edward H. and Martha F. Gerry Faculty Lectureship, and two Visiting Lectureship positions, one named in honor of Edward and one in honor of Martha, all as part of the endowment for the Watson School of Biological Sciences.

More recently, Martha provided support for the Watson School Fund for Innovative Graduate Education, CSHL's Women's Partnership for Science initiative, and the continuing CSHL Capital Campaign for the new research buildings on our Hillside Campus expansion project.

Martha lived in New York in the summer, but she was a regular visitor to Saratoga, New York for the horse-racing season there, to her stables in Kentucky, to her plantation in northern Florida, and to her beachside home on Jupiter Island. Wherever she was, she kept in touch with the programs at Cold Spring Harbor Laboratory and continued to do her homework. We are so grateful for her support and time that she shared with us on Long Island as a neighbor in Mill Neck. In many ways, she will always be here. I often find myself sitting in the Gerry Conference Room of the Marks Building, which we dedicated to Martha for her key role in realizing the Marks Laboratory for brain imaging. I'm glad to see her name and picture on the wall, even though I know it was never her goal to have things named after her. It is fitting that the picture is of Martha with her famous horse Forego. It's always a pleasure to be in her company in that room, where members of the CSHL community will continue the conversations about science that she so vigorously supported and where we teach the new class of Watson School students each year.

Bruce Stillman



William Edward Murray, Esq. (1926–2007)

William Edward Murray, Esq., of both Charleston, South Carolina, and New York City, deployed his high intelligence, broad vision, and keen liking for innovative medical research to greatly broaden the scientific aspirations of Cold Spring Harbor Laboratory for more than 20 years. Educated at The Citadel and the University of South Carolina before serving as a Naval Lieutenant in the Pacific during World War II, Bill obtained his first law training at the University of South Carolina. After moving north to Harvard, he earned a Master of Law (LL.M.), being then inducted into Phi Beta Kappa. At New York University School of Law, he completed his legal training with a Doctor of Judicial Science (J.S.D.). In 1949, he commenced his career at the Chief Council's Office of the Internal Revenue Service, where he acquired an intimate awareness of the tax codes for estates and trials, knowledge that he used to great profit in his subsequent career as one of New York City's leading tax attorneys. At the same time, Bill became an impassioned investor in commercial real estate, founding in 1963 the East Bay Company of Charleston, long serving as both its CEO and Chairman. The company has been instrumental in the revitalization of many historic sections of Charleston, a city that he so deeply loved.

Although I knew Bill largely through his desire to promote biomedical research, his philanthropic interests were much broader, including many years on the Board of Marymount Manhattan College, where he long served as its Chairman. In South Carolina, his interests were even more diverse, the Samuel Freeman Charitable Trust making gifts to Allen College as well as the University of South Carolina, the College of Charleston, and the Medical University of South Carolina in Charleston. Bill served as a trustee of Educational TV of South Carolina, Director of the American Intercultural Student Exchange, and an impassioned supporter of the East-West Institute that fostered exchanges between Eastern Europe and the United States.

I was first introduced to Bill when one of our trustees, Mary Lindsay, arranged for him to visit the Lab in the fall of 1984. Mary's unmarried first cousin, Samuel Freeman, had died in 1983, leaving Bill as the principal trustee of the Samuel Freeman Charitable Trust. In his will, Samuel Freeman expressed his wish that St. Pauls School and cancer research be major beneficiaries of his fortune, which included the New Jersey Black River and Western Railroad (a tourist railroad). Bill already was directing some of the Trust's monies to the Cancer Research Institute of New York, in particular allowing Sloan-Kettering's highly intelligent Lloyd Old work to develop vaccines against cancer. Mary, knowing that the Trust's monies were not small, thought that Bill would also help us to expand our cancer research program. Already, Freeman monies were allowing St. Paul's complete a new building for science, and I volunteered to see it in operation, a visit that occurred the following February.

Never slow-moving, Bill immediately liked how we were progressing in our cancer research, and soon after the end of our lunch at Robertson House, he made a commitment of $15,000 for equipment for monoclonal antibody research within our newly completed addition to James Laboratory (soon to be called Sambrook Laboratory). We were then in the process of completing our new 360-seat auditorium, made possible by a major gift from Oliver and Lorraine Grace. Long a friend of Oliver through their joint involvement with the vaccines effort of the Cancer Research Institute, Bill persuaded their respective trustees to use $350,000 of Samuel Freeman Charitable Trust monies and $50,000 of Oliver S. and Jennie M. Donaldson Charitable Trust

monies (for which he also was the principal trustee) to create the Samuel Freeman Computer Center in the lower floor of Grace Auditorium. Through its six Sun workstations, the Lab now had a dedicated facility for storing and retrieving DNA sequences as well as the use of X-ray and computer graphics techniques to solve the structures of cancer-causing and DNA-binding proteins.

Soon after, the Lab commenced its largest ever fund-raising drive (Second Century Campaign) to build a new dormitory (Dolan Hall) and a Neuroscience Building (Beckman) in the clearing above Grace Auditorium, then occupied by the Page Motel and adjoining cabins. Not wanting its construction to be held up until sufficient building funds were on hand, Bill, in 1987, arranged for the Samuel Freeman Charitable Trust to donate $200,000, allowing our architects, Centerbrook of Essex, Connecticut, to expedite the preparation of the final (construction) architectural drawings. Through that gift, the 1989 groundbreaking of the Neuroscience Center occurred some 8 months sooner than it could have without Bill's help.

The Samuel Freeman Charitable Trust returned to its focus on cancer research with a 1989 gift of $1 million to bring into existence the Samuel Freeman Laboratory of Cancer Cell Biology. To be expended over 3 years, these monies permitted our highly innovative McClintock Lab cell biology effort to use monoclonal antibodies against cytoskeletal proteins and two-dimensional electrophoresis gels to study how proteins coded by oncogenes transform normal cells into their cancer equivalents. Freeman funds allowed our more than motivated scientist, Bob Franza, to generate the most highly cited 1989 paper in all of cancer research. This paper revealed how external growth signals lead to the emergence of highly specific DNA-binding proteins that turn on the reading of genes, promoting cell growth and division. Two years later, the Oliver S. and Jennie R. Donaldson Charitable Trust, following Bill's wish, made a grant of $250,000 to our Moscow-born Grisha Enikolopov to develop inhibitors of key molecular promotion of cancer cell growth.

Bill, by then, was also directly helping Soviet science, seeing that $50,000 of Freeman funds went to Professor Gary Abelov's Laboratory of Immunochemistry at the Cancer Research Institute in Moscow. Earlier, his creation of an exchange program between Soviet and American immunologists led to his 1992 election to the Russian Academy of Science. Freeman monies to the East-West Institute led not only to the exchange of visits between South Carolina and Moscow, but to Bill's increasingly frequent visits to the Soviet Union. His visit to the pristine Lake Baykal in Siberia led to renovation monies that put again to sea "The Samuel Freeman" marine research vessel.

After I became Cold Spring Harbor Laboratory's President, Bill became a founding member of my President's Council, attending in May 1995 its meeting entitled *The New Human Genetics: Implications for Society*. Bill joined our Board of Trustees at its annual 1995 meeting, serving for two 3-year terms. From then on, as an Honorary Trustee, he continued to attend many of its meetings.

Bill's ever-increasing interest in the neurosciences led to the Samuel Freeman Charitable Trust providing us in 1997 with a $1 million grant toward the construction of the Samuel Freeman Building for Computational Neuroscience. Dominating the entryway to its William E. Murray seminar room is a powerful life-size photo of Bill in his office. Until a year before its dedication, Bill enjoyed fine health and the companionship of his loving partner of 10 years, Helen Flynn. Then he suffered a slight stroke that, to our great relief, in no way kept Bill from appearing characteristically animated at its dedication ceremony in May 2000. Sadly, this first sign of mortality was a harbinger of further illnesses to come. All too soon, he began to show symptoms of Parkinson's disease that increasingly limited his freedom to move his affairs forward. My close neurobiologist friend Rodolfo Llinas of New York University Medical Center was then starting to use high-power MEG imaging techniques to study brain dysfunction. To let him examine the molar and premolar cortexes of Parkinson victims, Bill deployed $100,000 of Freeman monies.

Increasingly, Bill and Helen were spending more and more of their time at their gracious Vendue Range apartment on Charleston Harbor, as opposed to their more formal flat at the Carlyle in New York City. Helen's arthritis and Bill's Parkinson's rigidity were better tolerated during the warm days of Charleston, rather than the cold days of New York. Bill's last visit to the Lab occurred in September 2005, when he and his youngest daughter Briana attended the dedication of our new Betty Livingston apartments at Upland Farms. The occasion also allowed us to honor Bill's 80th birthday on September 1 with an "80 Years of Distinction" booklet that showed pictures of Bill at his vibrant best. In late September, we again were with him at a gracious birthday party with his daughters Elizabeth, Pamela, and Briana at the Metropolitan Museum of Art.

At his funeral in Charleston, following his death on August 4, Liz and I remembered fondly the warmth and innate sparkle of Bill that so enriched the 20 years we had with him in our lives. Over and over, he saw that the Lab had the resources to bring my dreams to fruition. Although we never achieved our wish of sailing around Lake Baykal with him and Helen on "The Samuel Freeman" or celebrating together the White Nights of Saint Petersburg, they showed us Charleston at its best. I was privileged to receive the Honorary Degree of the Medical College of Charleston in 1987 and Liz its Honorary Degree in 1992 from the College of Charleston.

Cold Spring Harbor Laboratory will long cherish his memory.

James D. Watson

PRESIDENT'S REPORT

Science in the United States is at an important crossroad. Decisions taken in the next year or two are certain to affect the health and welfare of every American citizen as well as the ability of this country to continue to claim a leadership position in science and technology. Our challenges include improving the quality of science education and teachers, rekindling interest in science among our young people, easing immigration of scientists and engineers from abroad who continue to bring an indispensable vitality to U.S. research efforts, and most important restoring public support for basic science which has eroded measurably in recent years. Each of these issues affects the viability of the research enterprise here at Cold Spring Harbor Laboratory and at other leading scientific institutions across the country.

This year is a U.S. presidential election year and despite the importance of each of these issues, science has for the most part flown below the radar of public discourse. The American people certainly support science and scientific research in general, yet science does not enter into the national debate as much as it should. Not only Americans, but also all people in the world today face multiple threats to public health and to the natural environment. It is scientific research that provides us with the means to cope with these challenges, which are nothing short of existential. But science is not just a tool for addressing specific challenges. As an approach to understanding nature, the world in which we live and life itself, it steadily advances the frontiers of our knowledge and our capabilities and is thus a fundamental tool of human progress.

No one disputes that the products of the scientific enterprise generate benefits for everyone. But these accomplishments are often taken for granted. Nearly every American child and most adults now use the Internet. But, because the online world has become "second nature," we scarcely remember, much less actively contemplate, the wise policies that led our government to consistently support the computer science and physics research that made the Internet possible.

Major advances in physics, engineering, mathematics and computer science emerged from national efforts to compete in the space race that began in the late 1950s. At that time, politicians and public policy leaders, in consultation with scientific advisors, effectively articulated national goals that embraced science. Today, in the formulation of national policy, science has become an enterprise that is discussed mainly in times of crisis and scientists are low on the list of policy advisors consulted. Preparedness is a critical element for successful crisis management and it is therefore unfortunate that the societal commitment to funding a scientific infrastructure that is prepared to meet the challenges of society has waned.

As pointed out in an important 2007 National Academy of Sciences report entitled *Rising Above the Gathering Storm: Energizing and Employing America for a Brighter Economic Future*, the United States is falling far behind its major competitors in supporting education and research in the physical sciences, engineering and mathematics. If this "gathering storm" persists for much longer, the infrastructure for these sciences will be irrevocably damaged. The National Academy report made some important recommendations to our nation's leaders, but little has been done to implement them.

Although biology and medicine thus far have survived the reductions in federal government support that have cut deeply into the programs of their sister sciences, the fact remains that during the past 5 years Federal support for biomedical research has been reduced. In that span, the budget of the National Cancer Institute (NCI), for example, has not received any increase. In fact, it has been severely reduced if we take the effect of inflation into account. If the President's proposed budget for 2009 is implemented, the real purchasing power of NCI funding will have shrunk by 19% by the end of next year, relative to its value in 2004. What does this mean? The NCI Director has warned that the shrinkage threatens to make grants "meaningless," impairing the ability of basic and clinical investigators to achieve their stated goals with allotted resources that are constantly eroding over time.

The irony is breathtaking. This reduction in support comes at a time when real benefits in cancer research are emerging from work performed during the last two decades. Data from the American Cancer Society show that the cancer-related death rate per 100,000 Americans has been decreasing for the last 10 years, particularly for lung, colorectal and breast cancer. Such advances are not serendipitous but are rather the direct product of intensive basic and applied research over decades, made possible only because of a societal consensus regarding the importance of keeping financial support robust.

This is not the time to cut back on research. Rather, it is the right time to invest. New technologies, such as RNA interference (RNAi), mouse models for human cancer that accurately reflect the response that patients have to the illness, and genomic approaches to identifying new targets for therapy and diagnosis—all so prominently applied at Cold Spring Harbor Laboratory, as described in the pages of this report—are now being integrated into new strategies for cancer research and therapy. We are also now combining tools developed for cancer research to begin to unravel the mysteries of the brain and neurological diseases such as autism, schizophrenia, and Alzheimer's. At the same time, basic research remains our priority because it is the source of our future advances in both basic and applied research.

Reduction in funding not only limits what existing researchers can do, but it also discourages new scientists from pursuing science as a career. Undergraduate and graduate students very rapidly detect the anxious state of their professors when research funds become scarce. If a new generation of students in America is not thinking about a career in research, in short order the very fabric of American science will be compromised.

During the past 5 years we have experienced belt-tightening as have nearly all scientists across the country. Cold Spring Harbor Laboratory, however, has been able to survive and prosper due to the generosity of private donors who value our commitment to conducting outstanding research and education programs. Significant accomplishments in autism, schizophrenia, and cancer research have been supported either entirely or substantially by philanthropy. We are profoundly grateful for that support, but at the same time, we are obliged to raise a warning flag about public support for research over the long run.

Public support of science is vitally important because it complements the philanthropic support that enables great science to begin. For this reason, it is time to place science high on the list of public priorities. CSHL is in a position to challenge investigators—both those who work here and those who attend our meetings—to take an active role in public education and bring scientific discussion from laboratories into classrooms and living rooms in our community and across the country. It must be part of how we educate and train scientists today and in the future. We do this for our graduate students in the Watson School of Biological Sciences. This should be only the beginning of our efforts in this direction.

CSHL is also in a position to empower teachers and students of all ages by providing technological means with which they can enjoy easy access to real-time science. The Dolan DNA Learning Center (DNALC) is the key to the success of such an initiative. Through the DNALC and our educational and public affairs outreach, we must continue to broaden the population that we reach with our programs and resources.

If we at CSHL take a more proactive role in fulfilling our responsibility to society by pursuing these steps with fervor, we stand to succeed in continuing our own institution's leadership role in research. We will also be fulfilling our civic responsibility as scientists and citizens. If a concerted effort by scientists and scientific institutions to draw the public into what each of us has personally experienced as the wonder and promise of science is absent, we must ask: Can we realistically expect to overcome the national complacency that threatens the long-term stability of research activity in the United States?

CSHL has always been a leader and we have never shirked our responsibility to push science forward. As we continue to fulfill our research and education missions, let us not forget the importance of bringing the public along with us on our collective journey of discovery for the benefit of humankind.

HIGHLIGHTS OF THE YEAR

Research

Research performed at CSHL continues to have an extraordinary impact throughout the life sciences, most especially in the areas in which we have chosen to concentrate: cancer biology and genetics, neuroscience, genomics and bioinformatics, and plant genetics. A recent study—Thomson Scientific's *Essential Science Indicators*—placed CSHL in the top 1% of research institutions most frequently cited by scientific research papers. CSHL's scientists collectively ranked in the top 3% of their peer group, a strong indication of their influence on the entire field of molecular biology.

Such statistics are not the only measures of our effectiveness, but they speak unequivocally about the excellence of the research teams we have assembled on our small (although growing) campus. To convey more vividly the remarkable productivity of our programs, just a few of the dozens of important findings reported by CSHL labs during the last year are reviewed here.

Spontaneous Mutations in Autism

This year, a group of CSHL researchers that included Michael Wigler, Jonathan Sebat, and Lakshmi Muthuswamy published a landmark paper shedding light on the causation of autism. To the surprise of many, their work revealed that spontaneous mutations in the genome—gene copy-number variations—are a major cause of the disease. It has long been known that autism, like many other complex neuropsychiatric illnesses, has a strong genetic component. Yet most prior genetic studies have focused on families with more than one autistic child, perhaps skewing our understanding of the importance of inheritance. With generous support from the Simons Foundation, the CSHL team assembled genomic samples from more than 1000 individuals in 264 different families. By characterizing entire families—some with several autistic children and others with only one—and comparing these with controls, the team could determine whether specific sequences in a child's genome were identical to those in their parents' or whether their genomes contained variants that reflected copying errors.

M. Wigler

How common were copy-number variants (CNVs) in autistic children? The results were striking: CNVs proved to be ten times more common in the group representing families with only one autistic child. We now know that spontaneous mutations are a frequent cause of autism, which we can continue to think of as a *genetic* disorder, although not necessarily an *inherited* one. The team of investigators suspect that a higher-resolution analysis of the genome will reveal that as many as half of the autism cases are caused by spontaneous mutations. And they suggest that CNVs are likely to underlie other polygenetic disorders, including schizophrenia. More recently, the Sebat group found tantalizing new evidence that CNVs play a role in that devastating illness.

Pioneering the Small RNA Frontier

Work in Gregory Hannon's lab continues to expand the frontiers of knowledge about small RNAs and how they operate in complex networks to form RNA interference (RNAi) pathways.

G. Hannon

Greg and his team have been involved in this fast-changing area of research from its inception at the beginning of the decade. With the advent of research by Greg, Leemor Joshua-Tor, and other investigators on double-stranded RNAs and a previously unknown cellular machinery that "diced" and "sliced" them into short, single-stranded microRNAs (miRNAs) and short interfering RNAs (siRNAs), a wholly new class of RNA-based gene regulatory molecules came into view.

Greg's most recent work extends knowledge at the basic science level, while showing experimentally some of the applications that small RNA research is likely to have in years to come. This year, the Hannon lab, in collaboration with Senthil Muthuswamy's team, demonstrated that by manipulating miRNAs, including the tumor suppressor *let-7*, they could single out and repress stem-like cells in mouse breast tissue that are widely thought to give rise to cancer. Another team led by Greg, and including CSHL investigator Scott Lowe, identified a family of miRNAs (called miR-34) that enable a critical tumor suppressor network called the p53 pathway to fight cancer growth.

S. Muthuswamy

On the basic research front, Greg and colleagues have helped to reveal a new major class of small RNAs, called Piwi-, or piRNAs, which, unlike their small RNA "cousins," do not appear to arise from double-stranded RNA precursors. But like miRNA and siRNAs, piRNAs guide Argonaute proteins to silencing targets arrayed across the genome, via complementary base pairing. The Piwi clade has been found in mammals and flies; its presence is tightly correlated with the emergence of germ-line cells. Hannon has hypothesized in a recent paper that piRNAs provide a glimpse into the ancestral functions of RNAi, their emergence serving to counter the emergence—eons ago—of genomic parasites that threatened the earliest self-replicating genomes.

Unmasking a "Master" Tumor Suppressor

Naturally occurring tumor suppressive mechanisms have been the focus of work at the Laboratory for many years. This year, Alea Mills and colleagues solved a mystery in this area that has befuddled cancer researchers for decades. It had been known since the 1970s that the short arm of human chromosome 1 was often missing in many kinds of cancers, suggesting that this region contained a "good gene" that prevented tumors from forming. Yet attempts to pinpoint the gene by looking at human tumors turned up empty-handed.

Using an innovative method called chromosome engineering, Mills generated mice that were missing large fragments of DNA identical to the suspect tumor suppressive region in humans. Mice that lacked this region readily developed tumors, whereas mice with an extra copy of this region had excessive tumor suppression that made the cells stop dividing completely. Once they had pinpointed this powerful tumor suppressive region, Mills and her team determined which of the 52 genes in the region was responsible for preventing cancer.

A. Mills

They concluded that it was a gene called *Chd5*, the "master switch" of an extensive tumor suppressor network. When *Chd5* was deleted, the entire network collapsed and prolific cancer resulted. Alea and her team have already found that the gene is commonly deleted in an often fatal brain cancer called glioma, and they are now investigating whether it is responsible for other kinds of human cancers. Future efforts will be focused on exploring anticancer therapies based on modulation of *Chd5* activity.

Reversible Network Effects of Three Lung Cancer Genes

Cancer research that looks at one gene at a time is limited by the growing realization, made possible by the work of Scott Lowe and others, that cancers are often caused by networks of multiple interacting cancer genes. Mutations in these genes are widely thought to determine both response to treatment and clinical outcome in individual cases. This year, Scott Powers and David Mu, with collaborators at Memorial Sloan-Kettering Cancer Center, discovered that interactions of three adjacent genes found on human chromosome 14 can set off a potentially lethal chain of events that results in as many as one fifth of non-small-cell lung cancer cases. The genes—*TTF1*, *NKX2-8*, and *PAX9*—work together to reactivate what appears to be a pattern of gene expression normally present in the fetus, as lung tissue is being formed. Scott and colleagues not only identified the mutation that triggers this abnormal reactivation and demonstrated its causal role in human lung cancer, but also, intriguingly, demonstrated in model systems that by turning these genes off, the cancer can be stopped.

S. Powers

An Online Genome that Illustrates Genomic Variation

It is relevant to note that this year, CSHL launched a Web site enabling the general public to understand visually, as perhaps never before, a key dimension of genetic variation—the level of the single-nucleotide polymorphism, or SNP. With the online posting of Jim Watson's genome, it instantly became possible for anyone, using a plain Web browser, to view places where Watson's sequence is different from the "reference" human genome sequence published by the Human Genome Project. Easy-to-use navigation tools facilitate the viewing of genes and some of the common diseases associated with them.

Fixing an Editing Error to Treat a Neurodegenerative Disease

The process by which genes issue instructions to cells to produce specific proteins in specific amounts is intricate and usually very accurate. In a phase of this process called transcription, itself a marvel of complexity and precision, a strand of RNA is generated, which, in turn, is usually edited before leaving the nucleus to engage the ribosome, the cell's protein factory. Errors in the RNA editing process, called splicing, can lead to abnormal gene expression and in some cases serious disease.

Those suffering from spinal muscular atrophy (SMA), a neurodegenerative disease that is often fatal in children, are the victims of a splicing error about which Adrian Krainer and colleagues have learned a great deal. This year, Adrian's team published results of work that holds genuine promise for SMA patients. The team looked closely at how a mutated version of a gene called *SNM2* is expressed in the illness. In a part of the splicing process involving the precursor of the mature messenger RNA, the team focused on a deleted segment, exon 7, that is linked with the mutated gene's abnormal expression pattern in SMA. Using a class of molecules called antisense oligonucleotides, or ASOs, Adrian's team, in collaboration with a team at Isis Pharmaceuticals, was able to correct the splicing defect in cultured cells gathered from individuals with SMA. They now intend to test this therapeutic approach in mouse models.

A. Krainer

Another phase of the Krainer lab's work, which also has the potential to impact therapeutics, yielded results this year. They established that a splicing factor

called SF2/ASF, which they had previously described, can act to promote cancer. They demonstrated how the SF2/ASF protein, when produced in excess, can cause variations in the splicing patterns of many genes, one of which encodes a protein kinase that regulates cell growth and protein synthesis. A novel variant of this kinase, S6K1, can both cause and maintain cancer in cells.

An Inhibitory Neurotransmitter's Surprising Role in Neural Plasticity

Josh Huang and his team have made substantial progress in recent years in their efforts to characterize cellular and molecular mechanisms underlying the construction and plasticity of GABAergic inhibitory circuits in the mammalian brain. A prominent feature of GABAergic connectivity, which accounts for differential regulation of the input, integration, and output of principal neurons, is the targeting of different classes of inhibitory synapses to neuronal structures including spines, dendrites, somas, and initial axonal segments. This year, they made significant progress on the question of how GABAergic transmission is implicated in the regulation of plasticity, demonstrating how an environmental input can modify GABAergic neural circuitry.

J. Huang

This kind of work has direct implications in neurological and psychiatric illness such as autism and schizophrenia, which involve altered development and function of GABAergic circuits. To the surprise of many in the field, Josh and his team also discovered this year that the inhibitory neurotransmitter GABA itself has a major role in plasticity. They revealed that it serves as a trophic factor, with an impact on signaling that determines whether a synapse will be stable and whether axons will grow or retract.

It is important to mention that the great technical expertise in mouse engineering that makes possible the success of the Huang lab's research program has been recognized by the National Institutes of Health, which has awarded the group a major 5-year grant aimed at the generation of advanced genetic tools for study of the GABAergic system. Because the GABAergic network is very complex and heterogeneous, this will entail the generation of approximately 20 distinct mouse lines for each characteristic cell type.

A Novel Plant Gene That Calibrates Growth to Light

D. Jackson

In recent years, we have learned some surprising things about signaling in plants, including the fact that individual proteins and RNAs can act as the agents of mobile signaling. During 2007, David Jackson and his colleagues discovered a gene whose role in development brings to light a signaling mechanism that is scientifically intriguing and potentially of great commercial value. The gene in question, called *TILLERED1*, encodes a transcription factor that controls the growth of branching in grasses, a process called tillering. This gene, David reports, is particularly interesting because its transcription is calibrated to the sensing of light by the plant. He speculates that sensors in the leaves send signals, via a mechanism the lab is investigating, to the shoot meristem, the compartment within the plant in which stem cells that govern branching reside. Once understood, this signaling mechanism might be mobilized to boost the yield of plants such as switchgrass that are currently being used to make biofuels.

A New Partnership with Industry to Help Feed a Hungry World

To help meet the pressing needs of agriculture at a moment when the world's population is soaring toward the $7 billion mark, plant scientists at CSHL have joined forces with their counterparts at Pioneer Hi-Bred, the agricultural division of the DuPont Company. In an agreement reached this year that is certain to benefit both organizations, their formidable scientific acumen will be put to the challenge of helping farmers increase crop yields and grow plants specifically engineered to endure environmental stresses while protecting themselves from diseases and pests.

It is a partnership that departs in important ways from past agreements reached between academic research institutions and the private sector, many of which have been quite narrowly conceived. With thanks to John Maroney, Vice President, Technology Transfer, and his staff who worked closely with CSHL scientists, the agreement between CSHL and Pioneer encourages scientists in both organizations to communicate openly and share ideas, information, and research results. Rather than a single project, the teams will collaborate on several interrelated ventures over a 5-year period.

For Pioneer, it is a way to inject intellectual capital into an R&D enterprise that mostly focuses on applied work. For our plant scientists, it is a way to support a significant expansion of basic research while keeping one foot solidly planted in the "actual world," a world that sorely needs innovative solutions if nutritional and energy requirements are to be met in the decades ahead.

Forging a Collaborative to Define the Big Questions in Plant Biology

L. Stein

Just as our campus has served in past years as the setting for historic meetings at which plans were first sketched to sequence the human genome and the first plant genome, so it will witness in the coming months the inaugural meeting of a vitally important research collaborative that aims to define and address "grand challenge questions" in plant biology. CSHL and four research universities, led by the University of Arizona, will share a $50 million NSF grant over the next 5 years to launch the iPlant Collaborative, which will focus attention on research questions of global significance while at the same time orchestrating the development of an all-encompassing computer- and Internet-based infrastructure that promises to transform the way plant science is done.

D. Ware

Involving Rob Martienssen, Lincoln Stein, Doreen Ware, and Matt Vaughan of CSHL, the initiative's overarching objective is to bring plant science fully into the 21st century, as Rob has put the case, providing the technical and institutional means by which sharing and mutidisciplinarity can fully replace the outmoded single-investigator/single-institution research paradigm. It is a plan to provide plant science and scientists with the means with which to meet the extraordinary challenges to agriculture of the years immediately ahead, implicit in the steady rise in global populations, shrinking ecosystem diversity, and the prospect of significant global climate change.

Cold Spring Harbor Laboratory Board of Trustees

The Board of Trustees welcomed two new members at the beginning of the year: CSHL Association President Pien Bosch and American Express Executive Vice President and General Counsel Louise Parent.

We mourned the passing of two Honorary Trustees and long-time friends, Martha Farish Gerry and William E. Murray, Esq. Martha Gerry joined the Board of Trustees of the Laboratory in 1991, serving as Secretary of the Board from 1997 to 2000, and thereafter as an Honorary Trustee. Bill Murray was a member of the Board of Trustees of the Laboratory from 1995, serving as a Trustee until November 2001, and thereafter as an Honorary Trustee.

Many thanks to trustees Jacob Goldfield, Jeff Hawkins, and Douglas Morris, who began terms in 2000 and concluded their service this year. Mr. Goldfield, Managing Director, J. Goldfield & Co., participated in the Finance and Investment Committee from 2001 to 2004, the Investment Committee from 2004 to 2007, the Finance Committee in 2003 and 2005, and the Research Committee from 2004 to 2007. As a Scientific Trustee, Jeff Hawkins, cofounder of Numenta, Inc., served on the Research Committee from 2001 through 2007.

J.D. Watson

In recognition of his outstanding 40-year dedication to Cold Spring Harbor Laboratory, the Board of Trustees appointed James D. Watson Chancellor Emeritus and Oliver Grace Professor of Cancer Research Emeritus. Nobel laureate for the 1953 codiscovery of the double helix structure of DNA, Jim served as director of CSHL from 1968 to 1993, as president from 1994, and then as chancellor from 2003 to 2007.

Under Jim's leadership, CSHL rose to global prominence in cancer research and pioneered a broad range of genetic and biological education programs, including the doctoral program in the school that bears his name, the Watson School of Biological Sciences. He and his wife Liz have made invaluable contributions to this institution in so many ways, ranging from research and education to philanthropy, campus architecture, and art. We look forward to the Watsons' continued participation and support in campus life.

With Pien Bosch as president, the Cold Spring Harbor Laboratory Association (CSHLA) surpassed its 2007 fund-raising goal for the Annual Fund, raising nearly $1.3 million. Successful events that were organized throughout the year by CSHLA, with able help of our Development and Special Events Departments, included the Annual Meeting, the 9th Annual Jazz at the Lab, the Dorcas Cummings Lecture, a Major Donor Party hosted by Connie and Angelo Silveri, and the Women's Partnership Luncheon. Five new directors were named to the Association this year: Charles B. Goodwin, M.D., Lauryl Palatnick, David Peikon, Freddie Staller, and Fifield Whitman.

Research and Education Management

D.L. Spector

CSHL continues to strengthen the management of our research and education programs, which must evolve constantly to meet the challenges of contemporary science and society. This year, we appointed a new Director of Research, David L. Spector, who for the past two decades has focused his research at CSHL on advancing the ability of biologists to see the processes of transcription and translation in living cells. In addition to continuing to run his own laboratory, David's new charge is to oversee the entire scientific program and staff of more than 400. To support the Director of Research, we created the new position of Director of Research Operations, to which we appointed Sydney Gary, who previously had played a pivotal role as Assistant Director of the Banbury Center.

During the past year, the CSHL Grants Department has been transformed from a service division to a proactive Office of Sponsored Programs (OSP). Under the leadership of Dr.

Walter Goldschmidts, OSP now actively promotes our research and education mission by identifying and matching funding opportunities with CSHL research expertise and needs. A new team approach to facilitating and managing sponsored funding is accomplished by unprecedented collaboration with CSHL investigators, the Director of Research, and other CSHL departments. We have improved support and grant-related counseling for new investigators, enhanced budget monitoring and forecasting, and established a CSHL grant application archive. Grant-writing workshops were hosted throughout the year, and a new OSP intranet Web site with numerous resources available for the CSHL community was launched.

S. Lowe

The research management structure was further solidified by three outstanding Program Chairs in our focal research areas: Gregory Hannon, Bioinformatics/Genetics; Scott Lowe, Cancer and Molecular Biology; and Roberto Malinow, Neuroscience.

We also welcomed CSHL professor Leemor Joshua-Tor to a new leadership position as Dean of the Watson School for Biological Sciences (WSBS). Leemor will fuse her award-winning research into the molecular basis of nucleic acid regulatory processes with her expanded responsibilities, which include the doctoral program, the postdoctoral program, joint undergraduate Programs with Stony Brook University, the CSHL Summer Undergraduate Research Program, and the Partners for the Future Program for high school seniors. To provide additional support for the WSBS, the Assistant Dean position was filled by appointment of the former Assistant Director of the Dolan DNA Learning Center, Uwe Hilgert.

L. Joshua-Tor

New Markets for CSHL Education Programs

CSHL's program of scientific conferences and courses is one of the most recognized and respected in the biological sciences. Every year, more than 8000 researchers from around the world come to CSHL to participate in a wide range of meetings and courses. We are excited about the progress that David Stewart, Executive Director, Meetings and Courses, and his group have made over the last 2 years to bring a similar meetings and courses program to Asia.

At the end of 2007, we signed an agreement with the Suzhou Industrial Park (SIP), a new high-technology project located west of Shanghai and proximal to the 2500-year-old city of Suzhou. The largest cooperative project between the governments of China and Singapore, the SIP has become one of the fastest growing and most competitive development zones in China. Key to this success is the creation of a high-tech biotechnology and nanotechnology research and education park. As part of the agreement, CSHL will develop and operate an annual series of scientific conferences in a newly constructed complex within the SIP. We plan to open this conference program in 2010.

This year, the Howard Hughes Medical Institute (HHMI), which has supported courses at CSHL for more than 15 years, doubled its funding for the Lab's postgraduate education to $3 million during the next 4 years.

We are also pleased to be expanding our middle and high school education programs to New York City. Partnering with the New York City Department of Education, the Dolan DNA Learning Center (DNALC) was awarded a 5-year grant of $750,000 for precollege science education from the HHMI. This will enable CSHL to open a DNA Lab in a Harlem public

The DNALC brings interactive Web-based resources to its hands-on workshops and to teachers and students online

school, where we will train local teachers and give New York City students an opportunity to participate in laboratory DNA experiments.

The HHMI-funded program will supplement an existing DNALC "footlocker" program which currently provides training and footlockers full of lab equipment and supplies to 12,000 students in 60 school systems each year on Long Island. In addition, with leadership from DNALC Executive Director David Micklos, the talented DNALC staff began distribution of the world's first integrated set of RNA interference (RNAi) experiments which use the same technology that won the Nobel Prize in 2006.

Congratulations to Banbury Center staff and its Executive Director Jan Witkowski—who celebrated his 20-year anniversary with CSHL this year—for developing and executing 25 scientific meetings and nine courses on science and public policy. The breadth of topics covered at the Banbury Center is fantastic and this year was no exception. Highlights included "Protecting the Public Trust in Immunization" and "Retreat from Reason," which discussed how to bring rationality to public discourse.

Hillside Campus Update

Groundbreaking on the Hillside Campus expansion project began in 2005, but this year, we witnessed the most dramatic progress yet in this monumental capital project that will increase our research capacity in cancer, neuroscience, and bioinformatics by nearly 40%. At the beginning of the year, only the foundations for the six-building research complex were in place. By year's end, all of the internal piping and wiring were installed; the concrete superstructure was complete, exterior block walls were erected, and the roof structure and sheathing were in place, enclosing the buildings and ensuring that interior work continued through the winter months.

All the work on building structures could readily be seen at a glance as it progressed. Much less obvious was the enormous amount of work taking place inside the structures. Electricians and steamfitters worked hard, installing electrical and mechanical systems, including the two high-pressure boilers that will ultimately heat the six-building complex. These two areas were

Current construction of the Hillside Campus

by far the most complex components of the project, and the impressive progress made in these areas is essential to the timely completion of the Hillside Campus.

The final touches were also put on the new chiller plant, and much of the underlying infrastructure—drainage systems, electrical conduit and wiring, water and sewer piping—was installed underground.

The Nassau County Chapter of the New York State Society of Professional Engineers presented CSHL with the Project of the Year Award for the engineering and innovative environmental design of the storm water management system for the Hillside Campus. CSHL was recognized for achieving a balance between development and the environment. The unique and functional storm water management system not only is effective at protecting the surrounding ecosystems, but also provides a visually pleasing backdrop for the Laboratory.

We thank Vice President and Chief Facilities Officer Art Brings and the Facilities Department for all of these great results and the minimal disruption to our daily operations and quality of life on campus as the work has proceeded.

Awards and Honors

CSHL Professor and HHMI Investigator Greg Hannon was honored by two prestigious awards this year: The National Academy of Sciences 2007 Award in Molecular Biology for his extraordinary scientific achievement in molecular biology and The Memorial Sloan-Kettering Cancer Center's 2007 Paul Marks Prize for cancer research.

G. Hannon

Two CSHL postdoctoral students from Greg's laboratory received the prestigious Pathway to Independence Award from the National Institutes of Health. With this award, Jose Silva will be able to continue developing highly efficient RNAi libraries. Alexei Aravin's award was from the National Institute of Child Health and Human Development. This is a new NIH program, providing one of the few opportunities for postdoctoral fellows to receive both mentored and independent research support.

James D. Watson became the first individual to have his genome sequenced. The sequence was completed by collaboration between academic and industrial laboratories using a new rapid DNA sequencing method. Such personalized genome sequences will enable analysis of the details of human genome variation and will prompt needed public discussions about the use of such information.

Our new Dean of the Watson School of Biological Sciences, Leemor Joshua-Tor, was awarded the first Dorothy Crowfoot Hodgkin Award from the Protein Society in recognition of her exceptional contributions in protein science that have profoundly influenced our understanding of biology.

L. Trotman

Lloyd Trotman, who recently joined our faculty, was named a 2007 Rita Allen Foundation Scholar for his work on breast and colon cancer tumors. This program identifies promising leaders in research focused on the development of treatments and cures for cancer, cerebral palsy, or multiple sclerosis.

Lars Zender, a senior clinical postdoctoral fellow at CSHL, was awarded a $40,000 grant for his seminal contributions in the field of cancer biology and epigenomics. The grant was presented at the second annual “Cracking the Code with the Bear” research symposium sponsored by the Bear Necessities Pediatric Cancer Foundation in Chicago. In addition to Lars, CSHL postdoctoral fellows Danielle Irvine and Julius Brennecke were also selected to present their cancer research from a large pool of outstanding postdoctoral fellows across the country.

I was honored to receive the Curtin Medal for Excellence in Medical Research from the John Curtin School of Medical Research at the Australian National University, where I received my doctorate in 1979. In May, I also received an honorary degree and presented the commencement address at the Long Island University graduation.

At the 2007 Watson School of Biological Sciences Commencement Convocation on April 22, we conferred the Degree of Doctor of Philosophy to Darren James Burgess, Beth Li-Ju Chen, Catherine Yvonne Cormier, Claudia Edith Feierstein, Tomas Hromadka, Elizabeth Proby Murchison, and Dougal G. R. Tervo. Jonathan See-Ming Kui received the Master of Science degree. The Watson School awarded honorary degrees to Trustee Mary D. Lindsay, former CSHL Director Joseph F. Sambrook, and Nobel Prize winner Sydney Brenner.

Watson School Commencement Convocation. Former CSHL Director J.F. Sambrook receives his honorary degree.

CSHL was ranked among the most published and cited institutions by Thomson Scientific's *Essential Science Indicators*. In addition, *Genes and Development*, a publication of the CSHL Press, ranked sixth in the category of journals that publish high-impact research in molecular biology and genetics.

This fall, the CSHL Press published a groundbreaking textbook, *Evolution* by Nicholas H. Barton, Derek E.G. Briggs, Jonathan A. Eisen, David B. Goldsetin, and Nipam H. Patel., which for the first time integrates molecular biology, genome science, and evolutionary biology. CSHL Press Executive Director John Inglis and his staff of nearly 60 continue to innovate, most recently leveraging interactive digital technology. One excellent example of their effort to keep pace with society's increasing use of the Internet, editors at the Press have supplemented the newly released undergraduate *Evolution* textbook with a Web site (which even includes blogs) targeted for teachers and students who have adopted the text.

CSHL Press textbook *Evolution*

For the sixth consecutive year, CSHL received the highest possible rating from Charity Navigator for its financial organizational efficiency and capacity. According to Trent Stamp, President of Charity Navigator, "with only 1% of the charities rated receiving six consecutive four-star Charity Navigator evaluations, Cold Spring Harbor Laboratory outperforms most charities in fiscal responsibility."

The critical job of maintaining the highest standards of financial responsibility is ably performed at CSHL by Lari Russo, Vice President, Finance and Comptroller, and her team. These standards distinguish CSHL as an institution prepared to meet the expectations of public and private donors who demand accountability, transparency, and quantifiable results from their investments in nonprofit organizations.

Development

Under Charlie Prizzi, Vice President, Development, Cold Spring Harbor Laboratory's fund-raising efforts saw much success in 2007. An additional $20 million was committed to the Hillside Campus Campaign goal of $200 million. More than $40 million was raised in unrestricted and program support. On behalf of CSHL, our Board of Trustees, and our Development Department, I would like to acknowledge all those who helped us achieve our goals.

The Cold Spring Harbor Campaign

Capital, Endowment, and New Faculty and Laboratory Investment

We greatly appreciate the major donors who have generously contributed new gifts and pledges of $100,000 or more to support the Laboratory's Hillside Campus Campaign, including Dr. and Mrs. Bayard D. Clarkson, Mr. and Mrs. John P. Cleary, Mr. and Mrs. John H. Coleman, Mr. and Mrs. Norris Darrell, The Shelby Cullom Davis Foundation, the Estate of Norman Dorf, Empire State Development Corporation (New York State), the William Stamps Farish Fund, the Coleman Fung Foundation, Robert A. Gay, the Harrison Family Foundation, Mary D. Lindsay, Mr. and Mrs. Robert D. Lindsay and Family, the Estate of Elisabeth Sloan Livingston, Mr. and Mrs. David L. Luke III, Mr. and Mrs. Thomas D. McGrath, Jr., Mr. and Mrs. Alan Seligson, The Seraph Foundation, Mr. and Mrs. Theodore R. Stanley, Cynthia R. Stebbins, and the Roy J. Zuckerberg Family Foundation.

Education Programs

New gifts and pledges of $100,000 or more to support the Watson School of Biological Sciences, the Dolan DNA Learning Center, and other educational initiatives were gratefully received from Edward A. Chernoff, the Annette Kade Charitable Trust, The Jerome L. Greene Foundation, and the Doris M. and Peter S. Tilles Foundation.

Program Support

The Laboratory relies on private funding to maintain its innovative research programs. We appreciate new gifts and pledges of $100,000 or more from Mr. and Mrs. Donald Everett Axinn, The Mary K. Chapman Foundation, Kathryn Wasserman Davis, Dr. Leo A. Guthart, The Thomas Hartman Foundation for Parkinson's Research, Jo-Ellen and Ira Hazan, The Lita Annenberg Hazen Foundation, Joan's Legacy: The Joan Scarangello Foundation to Conquer Lung Cancer, The G. Harold and Leila Y. Mathers Charitable Foundation, The Don Monti Memorial Research Foundation, Manyu Ogale, V. Kann Rasmussen Foundation, The Sass Foundation for Medical Research, The Simons Foundation, Mr. and Mrs. Theodore R. Stanley, and the Swartz Foundation.

Breast Cancer Research Support

The Laboratory greatly appreciates the many individuals, foundations, and grassroots groups who provide the funding, outreach, and public awareness essential to maintain our cutting-edge breast cancer research. This year, CSHL was fortunate to receive support from Breast Cancer Awareness Day in memory of Elizabeth McFarland, Breast Cancer Help, The Breast Cancer Research Foundation, Clear Channel Enterprises/WALK 97.5, F.A.C.T. (Find A Cure Today), the Joni Gladowsky Breast Cancer Foundation, Glen Cove Cares, Elaine Hayes Special Effects Salon, Hearts for Cancer, Islip Breast Cancer Coalition, Long Beach Breast Cancer Coalition, Long Island 2-Day Walk To Fight Breast Cancer, L.I.A.B.C. (Long Islanders Against Breast Cancer), Manhasset Women's Coalition Against Breast Cancer, Pam and Pierre Omidyar, Sons of Italy, West Islip Youth Enrichment Services, and the Women's Insurance Network of Long Island.

The Robertson Research Fund

The Robertson Research Fund has been the primary in-house support for CSHL scientists for more than three decades. In 2007, it supported research in the labs of Grigori Enikolopov, Josh Huang, David Jackson, Leemor Joshua-Tor, Adrian Krainer, Rob Martienssen, Bill Tansey, Marja Timmermans, and Anthony Zador. Start-up research support was also provided by the Fund to four new investigators: Hiroyasu Furukawa, Raffaella Sordella, Lloyd Trotman, and Glenn Turner. In addition, the Robertson Research Fund continues to support the annual CSHL In-House Symposium and programs for postdoctoral fellows, graduate students, the laboratory seminar program, and faculty recruitment.

Library and Archives

We broke ground on a renovation and expansion project of the Carnegie Building that will allow CSHL's Library and Archives to serve our rapidly expanding campus and enable the continued collection of important materials in the field of molecular biology, genetics, and

Groundbreaking ceremony for the Library expansion. (*Left to right*) M. Pollock, W. Szybalski, J. Watson, H. Anand, and B. Stillman

biotechnology. The expansion is supported by CSHL donors, including corporate sponsor Genentech and CSHL alumni scientist and friend Waclaw Szybalski, Ph.D., Professor Emeritus of Oncology, University of Wisconsin-Madison.

The Archives completed a 2-year digitization project supported by the Josiah Macy, Jr. Foundation. A new database now allows us to convert our archival materials into digital format and to provide access through a Web-based interface. The Archives Advisory Committee, led by Nobel laureate and Chairman Rich Roberts, met for the second time since its formation in 2006. The committee decided to host a scientific meeting at CSHL in 2008 on the subject of the history of biotechnology. The Sydney Brenner Scholarship, endowed by Dr. Phillip Goulet, was awarded to noted scientist and biographer in history of science Jim Schwartz for his proposed project on Hermann J. Muller.

This year, we were fortunate to acquire the collection of prominent scientist and longtime CSHL Trustee Norton Zinder. The Medical Research Council donated to the Archives 136 notebooks of important research on *C. elegans* done by Sydney Brenner's laboratory. Under the leadership of Mila Pollock, the Library and Archives looks forward to organizing these new collections, along with contributions from pioneering scientist Charles Weissmann.

The year 2007 also marked the debut of the Library's CSHL Authors' Publications Database. The growing database of CSHL researchers currently contains more than 7500 citations from scientific journals spanning 1892 to the present.

Building Projects

It was another busy year for the CSHL Facilities Department, which undertook many simultaneous building projects during the year. The number, size, and ages of the Laboratory's facilities have necessitated a near-constant cycle of building and renovation.

As mentioned previously, the renovation and expansion of the Carnegie Building began in 2007. This project, scheduled with a very aggressive timetable, is slated for completion by the end of 2008. Not only will the project provide much-needed space in the CSHL Library and Archives, but it will also bring the historically significant structure up to current code and will be completely accessible to those will disabilities. Moreover, it will serve as the home for the new Genentech Center for the History of Molecular Biology and Biotechnology.

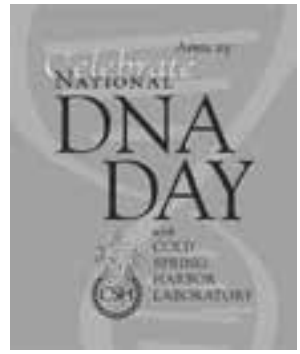
The Laboratory also undertook several projects to shore up the existing campus infrastructure. The last remaining section of high-tension electrical main was replaced, completing a project begun last year. To anticipate future needs, a water main was reinforced and a new gas main was connected to the campus. We also began a multiyear project to replace all building management systems with high-efficiency systems to control the heating and air conditioning. This project is anticipated to reduce power consumption in research buildings by 25% and is expected to lower energy costs by as much as $500,000 per year.

Other small projects include the renovation of the Darrell House, a single-family property formerly owned by a longtime neighbor of the Laboratory, the late Ms. Susan Rose. This house was renovated into faculty housing and is the last of three houses purchased by the Laboratory that encircle a historic garden designed by Frederick Law Olmstead. The Laboratory also completed the renovations of the James library, updating the room to meet current needs, and completed renovations in the James Laboratory. In 2007, the Laboratory renovated laboratories in Beckman and Delbrück to accommodate newly arriving faculty. The Osterhout house—formerly used as sabbatical housing—was converted to office space to accommodate library staff while construction takes place in the Carnegie Building. The Laboratory also continued its improvement of meeting facilities, building a reception office in Grace and renovating and expanding the nearly 60-year-old restrooms in the Bush Auditorium.

Special Events

National DNA Day

We celebrated the 5th anniversary of the congressionally designated “National DNA Day” on April 25, featuring special laboratory walking tours and festive signage on campus as well as throughout the village of Cold Spring Harbor. In addition to collaboration with the National Institutes of Health, our local partners in this celebration of DNA as part of our history and everyone’s future were the Cold Spring Harbor Library and Environmental Center and the Cold Spring Harbor Main Street Association.



CSHL celebrated National DNA Day April 25

Symposium

The 72nd annual Cold Spring Harbor Symposium celebrated the latest research on “Clocks and Rhythms,” welcoming more than 300 researchers from around the world with wide-ranging specialties in circadian clocks, aging, and developmental biology.

One of the traditional highlights of the Symposium is the Dorcas Cummings Memorial Lecture, this year delivered by Charles A. Czeisler, Ph.D., M.D., the Baldino Professor of Sleep Medicine and the Director of Sleep Medicine at Harvard Medical School, as well as the Chief of the Division of Sleep Medicine at Brigham and Women’s Hospital, Boston.

Jazz at the Lab

The 9th annual *Jazz at the Lab* on April 14 featured the Dena DeRose Trio with special guest Bob Merrill. The event raised more than $165,000 and was cochaired by CSHLA Directors Suzanne and Michael DiMaio and Maria Brisbane, and Wallace Henderson.

Concert for a Cure for Cancer

Violin prodigy Jourdan Urbach performed a benefit concert and reception on September 30. Proceeds benefited the Lustgarten Foundation for Pancreatic Cancer and CSHL.

Women's Partnership for Science

Nearly 200 women attended the Women's Partnership for Science benefit luncheon, held at the home of Mr. and Mrs. Daniel P. Davison on June 24, to learn about the most recent approaches to personalized treatment for breast cancer. The talk was given in tandem by CSHL investigator Mona Spector, Ph.D, and Jacqueline Bromberg, MD, Ph.D., Memorial Sloan-Kettering Cancer Center.

M. Spector and J. Bromberg

Gavin Borden Visiting Fellows

The 13th Annual Gavin Borden Visiting Fellow Lecture, in memory of the publisher of *Molecular Biology of the Cell*, was presented by Dr. David Baker on October 29.

Groundbreaking for Library and Archives

On October 12, more than 100 friends celebrated the groundbreaking for the renovation and expansion of the Carnegie Building, home to CSHL's Library and Archives and the new Genentech Center for the History of Molecular Biology and Biotechnology. At the podium, with shovels in hand, I was joined by the Mayor of the Village of Laurel Hollow Harvinder (Harry) Anand, Dr. Watson, Library benefactor Waclaw Szybalski, and Mila Pollock.

The Double Helix Medals Dinner

The Laboratory held its second Double Helix Medals Dinner on November 8 at the Mandarin Oriental, New York. Medals were presented to David Koch for Corporate Leadership and Richard Axel, M.D., and Michael Wigler, Ph.D., for Scientific Research. This special event, which raised more than $3 million for CSHL, was cochaired by Ms. Jamie C. Nicholls and Mr. Fran Biondi, Mr. and Mrs. Stephen M. Lessing, Dr. Arthur D. Levinson, Mr. and Mrs. Robert D. Lindsay, Mr. Sean McManus, and Mr. and Mrs. David M. Rubenstein.

CSHL and New York Public Library cohosted a public lecture series called Science Soirées

Science Soirees

CSHL and The New York Public Library's Science, Industry, and Business Library (SIBL) sponsored a series to help nonscientists better understand the scientific concepts fundamental to understanding

Double Helix Medals Dinner participants. (*Left to right*) E. Lauder, D. Koch, A. Hayes-Dale, and J. Koch

health and the well-being of society. According to CSHL researcher Partha Mitra, Crick-Clay Professor of Biomathematics and series moderator, "The idea is to expose nonscientists to fundamental scientific concepts that could affect their lives and provide them with a foundation for informed decisions and further exploration."

February 1
Tim Tully, Professor, Cold Spring Harbor Laboratory: *The Future of Memory: The Biochemistry of Memory and Its Future Enhancement.*

March 12
Nicholas D. Schiff, Associate Professor and Associate Attending Neurologist, Weill Medical College of Cornell University: *Regaining Consciousness: Recovery from Severe Brain Injury.*

April 9
Jeff Dangl, John N. Couch Distinguished Professor of Biology, The University of North Carolina at Chapel Hill: *Genetically Modified Foods: Revolutions in Plant Science and the Controversies They Spur.*

May 1
Robert L. Kleinberg, Senior Research Scientist and Scientific Advisor, Schlumberger Research: *Energy Sources of the Future: The Arctic National Wildlife Refuge, Global Warming, and National Security*.

June 11
Partha Mitra, Professor, Cold Spring Harbor Laboratory: *Learning to Sing: Birdsong, Baby Talk, and the Origins of Language.*

CSHL Public Lecture Series

March 13
Don Axinn, *What You Always Wanted to Know About Poets' Lives, Their Odd Behavior, and Their Creative Process but Were Afraid to Ask.*

May 15
Gregory Hannon, HHMI Investigator and Professor, Cold Spring Harbor Laboratory: *Recent Progress in Cancer Research.*

June 21
Portia Iversen, Cofounder of the Cure Autism Now Foundation and the Autism Genetic Resource Exchange: *An Evening About Autism.*

September 25
Avner Hershlag, Director of North Shore Hospital's Preimplantation Genetic Diagnosis program: *Custom-made Babies: Fact or Fiction?*

October 23
Robert Malinow, Professor, Cold Spring Harbor Laboratory: Recent Progress in Neuroscience Research.


R. Malinow

Public Concerts

April 21
Chu-Fang Huang, piano

April 28
Yousun Chung and Teddy Robie, oboe and piano

May 5
The Parker String Quartet, violins, viola, and cello

May 19
Liza Ferschtman and Inon Barnatan, violin and piano

September 8
Wendy Warner and Irina Nuzova, cello-piano duo

September 29
Peter Orth, piano

October 13
Jean-Frederic Neuburger, piano

Exhibits

Photographer-in-Residence Max S. Gerber exhibited his work in a show titled "Faces of Science," in the Bush Auditorium from July 13 through July 29.

Laboratory Employees

New Staff

We welcomed Lloyd Trotman as an Assistant Professor in the CSHL Cancer Center, where he will continue to research the role of PTEN "dosage" in breast and colon cancers. Lloyd received his Ph.D. from the University of Zurich and did his postdoctoral fellowship at Memorial Sloan-Kettering Cancer Center. At MSKCC, he and his colleagues found that removal of only one copy of PTEN was sufficient to induce cancer in a mouse model of prostate cancer and that removing both copies prevented the growth of cancer cells.

Dagnia Zeidlickis joined the CSHL staff as Vice President, Communications, with a mission to further our success in educating the general public about CSHL's biomedical research

and education initiatives and accomplishments. Dagnia brings international experience in public and government relations to the Public Affairs Department. Joining her later in the year was Jim Bono, our new Director of Public Affairs. His media experience and understanding of our private and public sector audiences locally and throughout the state will be an asset to our institution.

A. Zador

Promotions

Congratulations to Anthony Zador who was promoted to Professor and Director of the Swartz Center for Computational Neuroscience. We also commend Senthil Muthuswamy who is now Associate Professor, Adam Kepecs who is Assistant Professor, and Matthew Vaughn who was promoted to Research Assistant Professor.

Departures

During the course of the year, several faculty members took on new challenges at other institutions. Lilian Clark, our former Dean of the Watson School of Biological Sciences, became the Executive Director for Research Operations and Funding at Cancer Research U.K. I thank Lilian for the enormous amount of work she did to help establish and eventually run the Watson School. The success of the School is a reflection of her talent and capabilities.

Professor Tasuya Hirano moved to the Riken Discovery Research Institute in Japan. Two CSHL researchers joined Howard Hughes Medical Institute's Janelia Farm: Professor Karel Svoboda, who continues his relationship with CSHL as an adjunct professor; and Associate Professor Dmitri Chklovskii, who also continues here as an adjunct associate professor. Similarly maintaining ties to CSHL, Professor Tim Tully remains an adjunct professor while assuming the new role of Chief Scientific Officer at Dart Neuroscience, a company that he cofounded. Wolfgang Lukowitz is now an adjunct assistant professor at CSHL, with a new position as assistant professor at the University of Georgia in Athens. Michael Myers, who was an assistant professor at CSHL, is now with the International Centre for Genetic Engineering & Biotechnology in Italy.

Community Outreach

CSHL employees continue to actively participate in local and national community service events, including Lab-wide Blood Drives in February, August, and December, the American Cancer Society's Daffodil Days in the spring, and both the John Theissen Children's Foundation Toy Drive and the Suffolk County Toy Drive in December. Every year, CSHL is proud to sponsor the Cold Spring Harbor Library and Environmental Center kickoff festivities for the children's summer reading program.

We also continue to open the CSHL campus to guided walking tours, which are open to the public most weekends from spring through the fall. Thanks to our specially trained team of tour guides, who include CSHL students and postdocs, and the Meetings and Courses team that administers this program. They open our doors to new friends and provide tour participants with hands-on insight into the fantastic campus that we have built.

Looking Forward

2007 was another remarkable year in the history of CSHL. Our research, education, and administrative staff continue to surpass expectations. With Dill Ayres as our Chief Operating Officer, this staff—now more than 1000 strong—put CSHL at the forefront of biomedical science in so many respects. During this year, we have all worked hard to prepare for the future—a future brimming with the potential of more breakthroughs. I appreciate all of the efforts of our employees and thank Katie Raftery, Vice President, Human Resources, and her Department for continuing to help make CSHL such a great place to work.

I often marvel at the fact that despite our long and rich history and despite our significant growth, CSHL remains nimble in its ability not only to react, but also to anticipate change. The world around us is changing fast in so many ways—the economy, the political climate, the physical environment, societal norms. Evolution is a concept that we as scientists embrace. We as an institution have also embraced this concept and are stronger for it. Kudos to the Trustees, faculty, staff, and supporters for your help in making sure that CSHL continues to stay ahead of the curve.

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

CHIEF OPERATING OFFICER'S REPORT

The year 2007 marked a period of steady progress across the full scope of Cold Spring Harbor Laboratory's operations, made possible by the effort and dedication of our faculty, students, and administrative staff.

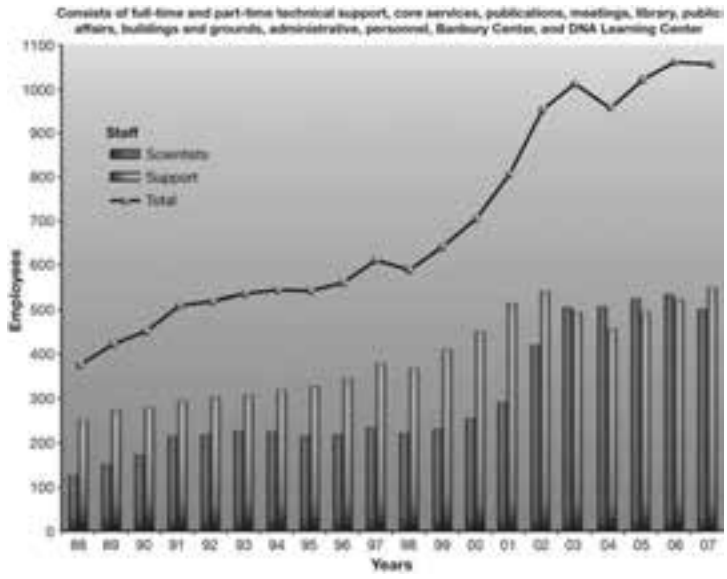
The most outwardly visible indication of change is the ongoing construction of the new research buildings on our "Hillside Campus." During the course of the year, the exterior of the buildings emerged on the landscape at a rapid pace. At year end, all six buildings were up with roofs on and windows installed. Given the magnitude of this project—100,000 square feet of new space under construction—it has been remarkable how little disruption there has been to the quality of life on our main campus. This is a credit to both the leadership of our own Facilities Department and the diligence of the general contractor. In the coming year, we will see the exteriors and interiors of the buildings near completion, with some of the space ready for occupancy by early 2009. The restoration and reforestation of the site will continue in earnest next spring, resulting in what we are confident will be a handsome and architecturally consistent addition to our beautiful campus. It is gratifying to be able to report that, as of year-end, the project is progressing on time and on budget.

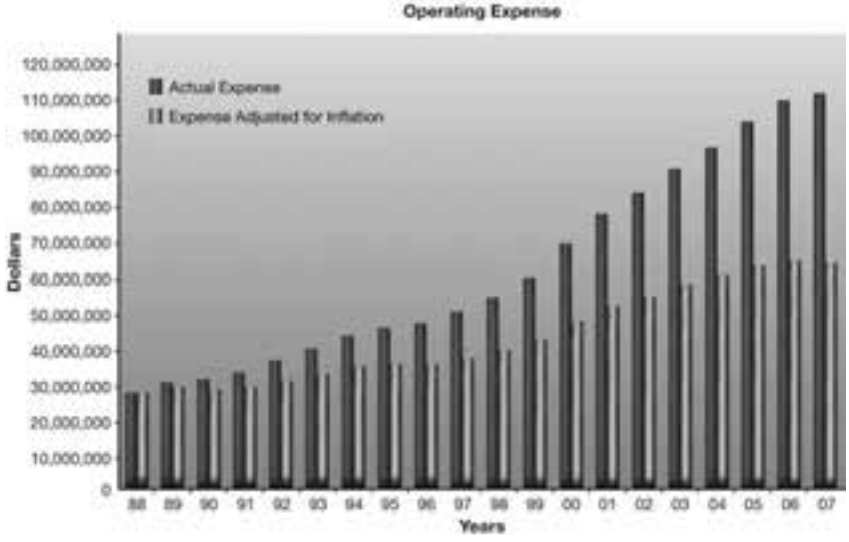
To support this expansion, CSHL announced in 2006 its largest ever capital campaign. The $200 million campaign goal includes funds earmarked for construction costs, faculty recruitment and start-up, and endowment. Thanks to the efforts of our Development Office, our Trustees, and the many generous supporters of the Laboratory, we expect to reach the goal and complete the campaign by the end of 2008.

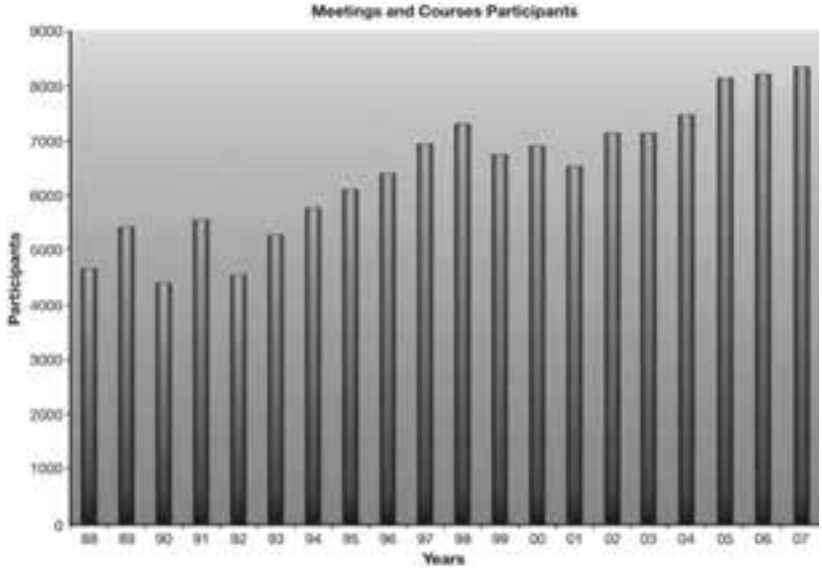
When the campus expansion was in the planning stage 6 years ago, there was little reason to assume that federal funding for research would contract as it has. At the same time, certain areas of operating expense such as energy, insurance, and health care have proven difficult to control while cutting-edge research is becoming an increasingly expensive endeavor. Because we see no short-term change to this altered environment, important initiatives have been undertaken in response and have generated positive results. Management is exploring appropriate corporate research support agreements such as one entered into this year with DuPont Pioneer to work in collaboration with our plant genetics group.

The effort to increase private support for research has also been very successful thanks, most notably, to the Simons Foundation's support of our autism program and to the Stanley Foundation support of a program for schizophrenia and cognitive disorders. To ensure that we are doing everything possible to maximize our share of the federal pool of funds, Dr. Walter Goldschmidts joined the Laboratory in 2006 from the National Institutes of Health as Executive Director of Sponsored Programs. He is working very effectively with our new Director of Research Dr. David Spector and our investigators to both increase grant submissions and manage research budgets proactively.

In addition, the Administration continued to vigorously manage overhead and operational expenses. Difficult decisions were made in 2007, the toughest of which was to freeze salaries and limit new administrative hires. We ask a great deal of our dedicated staff, and it was particularly painful to not offer raises at a time when property taxes and fuel costs are increasing so dramatically in our geographic area.
For the fiscal year ended 12/31/07, the Laboratory operating budget reached $115.5 million—a 3.2% increase over the prior year. Total assets were $639 million—an increase of 10%. Our objective each year is to balance our operating budget after full depreciation expense, a $6.5 million item in 2007 or, at a minimum, to be cash-neutral after netting depreciation expense against spending on capital improvements. Our ability to achieve the latter goal was greatly enhanced again this year by the success of our Cold Spring Harbor Laboratory Association Annual Fund and the Double Helix Medals Gala in Manhattan, which together raised nearly $4 million in unrestricted funds. As our sources of funding shifted toward







increased corporate and private support, we also relied more this year on internal funds by slightly increasing what has historically been a conservative annual spending rate on our endowment.

The endowment fund achieved positive return on investment for the fifth consecutive year, surpassing $300 million in year-end market value for the first time. Accounting for the effects of new endowment gifts, annual spending, income, and appreciation, the fund has grown to this level from its establishment in 1973 with an $8 million gift from the Charles Robertson family. Total return for the calendar year 2007 was 9.1%, well in excess of the S&P and other benchmark indices. Although these are certainly positive results, the markets and the economy became very choppy in the last two months of the year and we cannot necessarily assume positive returns in 2008.

We have our work cut out for ourselves in the coming year. A top priority will be to continue to be proactive in developing private sources of funding for research and start-up expenses. The recruitment of new investigators for the expanded campus and the planning for bringing the new buildings on line will require continued energy and focus. Integration of new personnel and facilities is challenging and demands careful planning and execution. Overhead will increase substantially so it is imperative that expenses continue to be managed diligently.

Our talented and prominent Board of Trustees continues to provide valued leadership and support to the Laboratory. We express our gratitude to three retiring Trustees in 2007—Jacob Goldfield, Jeff Hawkins, and Doug Morris. Elected to the Board this year were Louise Parent and Cold Spring Harbor Laboratory Association President Pien Bosch.

More than ever, this year, it is important to recognize the effort and dedication of our 1000 employees. In a year where they have been asked to give more than they got, the Cold Spring Harbor Laboratory staff is most deserving of our praise and gratitude.

Dill Ayres
Chief Operating Officer

LONG-TERM SERVICE

(*Top row*) Jan Witkowski, Inez Sialiano, Stanley Schwarz, Lisa Bianco, Bruce Stillman, Patricia Barker, John Inglis, Barbara Peters, Randal Jones, David Spector. (*Bottom row*) Judy Smith, Sadie Arana, Simone Salghetti, Barbara Zane, Julie Ehrlich, Patricia Bird, Nancy Hodson

The following employees celebrated milestone anniversaries in 2007:

35 years	Terri Grodzicker
25 years	Patricia Bird, Arthur Brings, David Micklos, Clifford Sutkevich
20 years	Sadie Arana, Patricia Barker, Lisa Bianco, Joan Doxey, James Duffy, Julie Ehrlich, John Inglis, Barbara Peters, Stanley Schwarz, Inez Sialiano, Patricia Urena, Jan Witkowski
15 years	Robert Collins, Gregory Hannon, Nancy Hodson, Randal Jones, Richard McCombie, John Pisciotta, Simone Salghetti, Judith Smith, Mary Smith, William Tansey, Leslie Wenzel, Curtis Williams, Barbara Zane, Yi Zhong

CHANCELLOR'S REPORT

Schizophrenia remains a diagnosis that elicits fear and despair among individuals whose lives it envelopes directly or indirectly. Approximately 1% of every human population, with no known exception, lives with brains that inappropriately respond to their social milieu, dominated by the "positive symptoms" of delusions, paranoia, voices, and visions that have no basis in reality. Equally devastating is the fact that most schizophrenia victims have a diminished capacity to organize their thoughts and rationally plan their future courses of action. These "negative symptoms" distinguish schizophrenia from its equally common relative bipolar disease (manic depression). With both diseases, medicines, although far from perfect, routinely bring most victims out of dangerous psychotic moments. For most who suffer from bipolar disease, such antipsychotic and subsequent mood-stabilizing medicines, available since the late 1950s, restore the capacity for almost normal life—either attending school or holding rational, thought-demanding vocations. However, such medicines unfortunately have no impact on the negative symptoms of most victims of schizophrenia. As they approach old age and their families lose the ability to care for them, they all too often transition into the homeless outcasts of human society.

At the heart of the despondency that schizophrenia evokes in those who care for its victims is our lack of knowledge of its ultimate underlying causes. No evidence exists for causative infectious agents, and our most useful clues come from comparison of the incidence of schizophrenia in identical versus fraternal twins. Its much higher concordance in identical twins argues for inappropriate genetic instructions at the heart of much of serious mental disease. Even this "fact," however, is not easily accepted, with many believing that mental disease results more from the stressful conditions of modern society than from the possession of "bad" genes.

That such "nurture" as opposed to "nature" causation explanations remain prevalent even today owes much to the failure of psychiatric geneticists to demonstrate the existence of even one gene located at a precise chromosomal location that carries susceptibility for schizophrenia and/or bipolar diseases. When successful gene mapping finally took off in the mid-1980s and the causative genes for Huntington's disease, cystic fibrosis, and breast cancer were given precise locations on chromosomes 4, 7, and 17, no equivalent "eureka" moments occurred for psychiatric diseases. Unclear then and still today was whether the "bad" genes behind schizophrenia and bipolar disease are disease-causing only when inherited from both parents (recessive genes) or whether a "bad" gene from only one parent (dominant gene) is necessary. And although identical twins are much more likely to be dually afflicted than fraternal twins (50% vs. 10%), why identical twins are not 100% concordant remains mysterious.

Keeping psychiatric genetics moving in the unrewarding 1990s was the knowledge that the Human Genome Project was on course and scheduled to be completed soon after the beginning of the 21st century. With a completed human DNA sequence on hand, there would be virtually infinite numbers of DNA sequence variants for use as genetic markers. It is still too early to assess whether the long-sought nirvana of classical psychiatric genetics is within its grasp. The Wellcome Trust's first big associative study for manic depressive disease was not reassuring. No one gene or small group of genes were discovered to be major players behind that illness' disruptive cycling between manic and depressive behavior. Although

we know now that several well-defined genes—neurogulin, disbindin, and DISC (disrupted in schizophrenia)—contribute to schizophrenia in some families, none found so far seem to affect more than a small percentage of victims.

The best way for psychiatric genetics to proceed may not be through further large-scale genetic association studies, but by direct examination of the DNA of mentally ill individuals. Within a decade, the science basis should exist for routinely examining the DNA of the mentally disturbed to determine which genes are at the root of their illness. Helping us to realize this goal will be the ever-decreasing cost of DNA sequencing. Although the cost of establishing the first complete human genome was in the several $100 million range, subsequent genomes come much cheaper. Craig Venter's personal genome, generated in 2007 using the same capillary sequence techniques that had generated the first (2003) genome, was likely in the $10 million-plus range. Such mega costs, beyond the reach of almost all humans, will soon be much reduced by use of several powerful new sequencing technologies. For my personal genome of June 2007, using *454 Life Sciences* technologies, Roche expended slightly more than $1 million. During the next decade, the cost of personal genomes is likely to fall to that of a Chevrolet-like economy automobile.

Until then, the most cost-effective way to discover the genes responsible for serious mental illness will be the examination of individual human genomes for the presence of changes (polymorphisms) in the copy number of the approximately 23,000 different human genes. Normally, each of us has two copies of each of our genes (one each from our father and our mother). During germ-cell formation, however, illegitimate genetic recombination events continually create mutant chromosomes lacking a specific gene or containing multiple copies of the gene. Now, it seems likely that gene-copy-number changes, because of their much larger sizes, are more likely individually to lead to diseases than the changes of one base pair to another (e.g., AT→GC) along human chromosomes. Today, our best guess is that each newly born individual contains 20–40 copy-number polymorphisms and some 300–400 single-base-pair replacement insertions or deletions not present in either parent. At present, the cost of finding all of an individual's copy-number changes using DNA chips is 1000-fold less expensive than using DNA sequencing to additionally find the many single-base-pair changes not present in the parents' DNA.

Before the human genome sequence was established, several microscopically visible insertions and deletions (many million base pairs long) were shown to cause 1–2% of both autism and schizophrenia. But because they invariably involved multiple numbers of genes, the specific ones that cause autism or schizophrenia still remain unknown. More recently, using new human-genome-dependent DNA chip technologies, Mike Wigler's laboratory here at Cold Spring Harbor has shown that duplications and deletions of much shorter lengths are widely distributed over the 24 different human chromosomes. Through examining DNA from some 500 affected children, Mike and Jonathan Sebat discovered that autism-associated copy-number variations create autism at multiple chromosome locations. To determine the size of this universe of autism-causing genes, DNA from many thousands of such individuals must be examined. With the underlying "DNA chip" copy-number-finding technology likely to remain near $1000 per human genome, and with the National Institutes of Health effectively bankrupt, forthcoming large autistic gene searches will only be possible through enlightened mega-philanthropic gifts such as those now coming to Cold Spring Harbor Laboratory from Jim and Marilyn Simons.

Our success in discovering the DNA regions behind autism now encourages us to mount a similar effort to determine the likely large universe of genes causing schizophrenia and

bipolar disease. Already the first such search of some 500 victims' DNA, funded by the Lattner Foundation of Florida, has allowed CSHL investigators Jonathan Sebat and Sean McCarthy to discover among victims of schizophrenia gene-copy variants not yet observed in mentally healthy individuals. Most importantly, changes have already been observed in a large number of genes, most of which code for proteins found in nerve cells. As with autism, we will soon go on to examine the DNA of many thousands of schizophrenia and bipolar victims. The funds for much of this effort are already in hand thanks to the multiyear many-million-dollar gifts from Ted and Vada Stanley.

Mike Wigler has gone on to conclude that most of the "bad" genes behind autism need be present in only one copy in order to be expressed in boys. In contrast, most girls are resistant to single copies, becoming carriers of autism—half of whose sons will be autistic. Why most girls are resistant remains a total puzzle. We remain equally ignorant of how schizophrenia runs in families. If one parent is schizophrenic, the probability of a child being schizophrenic is only 10%. A possible explanation for this apparently low penetrance is that many schizophrenia-predisposing genes must work in concert, say, in pairs, to be disease-causing.

Alternatively, DNA changes that occur during embryonic development may either diminish or raise the probability that a disease-causing gene from a schizophrenic father or mother expresses itself. "Epigenetic" chemical modification of the bases of DNA (e.g., methylation) occurring after birth are now frequently hypothesized to be at the heart of discordant-disease-pattern identical twins. Also to be seriously considered is whether much hereditary schizophrenia, like hereditary cancer, is a two-hit phenomenon with an inherited nerve-cell-specific recessive gene coming from one parent, being complemented by a second nerve-cell-specific mutation occurring early in embryonic development. According to this hypothesis, lineage-specific clusters of malfunctioning neurons lead to mental instability by interrupting the function of key brain circuitry.

That so many different genes when changed in dosage or base-pair content lead to autism, schizophrenia, or bipolar disease probably explains why these pernicious human afflictions are so common. With several thousand different proteins likely underlying brain function, its proper functioning can go wrong in so many ways. In contrast, the ability of adults to digest lactose remains absent from most human populations because changes in only several specific base pairs have the potential to generate lactase in adults. Whereas most newly arising genes leading to autism are quietly selected out of the human gene pool, this may not be true for genes leading to schizophrenia and bipolar disease, particularly if most single copies act recessively and are not selected against.

Although full-blown schizophrenia and bipolar disease are not thought to be limited to humans, it is not clear that we have observed sufficient numbers of chimpanzees or gorillas in captivity to so diagnose them. Soon, the ever-lowering costs of DNA sequencing will allow us to accurately measure the rates at which new mutations arise, not only in humans but also in our close animal relatives. Conceivably, human success in colonizing so much of the world's environment has selected for a higher mutation rate than found in the chimpanzee, whose life style may not have changed much over the past 5 million years. Although wildly speculative, this way of reasoning explains why "the good God above" has let so much inherent misery prevail today.

Arriving at an understanding of serious mental disease at the same advanced level that we now comprehend cancer is likely to be a long arduous task requiring at least several decades of sustained high-level science. As has been the case for cancer, an understanding at the genetic level will be essential for any rational approach to better medicines.

Fortunately, the tools—both physical and intellectual—are on hand for ultimate success at the genetic level, but success will only prevail if we are able to expend large sums of money over many years.

As soon as they are discovered, each of many human genes likely to cause schizophrenia, bipolar disease, and autism must be studied in appropriate mouse models. Happily, there already exist several mouse models for the best known schizophrenia-associated gene, DISC, whose identification arose from elegant chromosome observation made during the past decade in David Porteous' lab in Edinburgh. DISC is already being used to search for new classes of antischizophrenic drugs.

Cold Spring Harbor Laboratory's current capacity to be a major player in finding the genes behind schizophrenia reflect the wisdom of its past Trustees in creating our Woodbury Genome Center for high-technology science, a building whose machines and computers are seemingly more conspicuous than its scientists. Our pursuit of mouse models for psychiatric disorders will proceed in the buildings and animal facilities of our new Hillside Campus scheduled to come on line about 1 year from now. Our decision to move ahead with this major building endeavor so soon after 9/11 was much to our Trustees' credit.

Forty years ago, I assumed the Directorship of this institution, believing that I could bring it back to financial and intellectual vigor through focusing its research program on understanding cancer. Emphasizing cancer, I thought, would in no way diminish our long intellectual commitment to pure science. To understand cancer cells, we would need to be simultaneously deeply knowledgeable about normal animal cells and understand how they change through embryonic development. Today, in deciding to focus much of the Lab's new resources on the cognitive disorders of autism, schizophrenia, and bipolar disease, we must understand the healthy brain and its extraordinary ability to perceive the outside world. In many ways, we remain as ignorant of how our brain allows us to learn, remember, and think as geneticists were about what the gene is and how it works before the Double Helix and the Genetic Code came on the scene. Extraordinary new worlds of knowledge are out there to be discovered.

I am now 10 days past 80. Focusing on making the important unknown known remains my way to stay alive.

James D. Watson
Chancellor

JOSEPH AND TITA MONTI

Cold Spring Harbor Laboratory is at the forefront of biomedical research and our dedicated investigators continue to expand the boundaries of science. Financial support from friends makes this all possible. This year, we mourned the passing of Joseph Monti and sadly remembered the loss of his beloved wife Tita Monti just a year earlier. Together, they built the Don Monti Memorial Research Foundation in memory of their son Don, who died of acute myeloblastic leukemia in 1972. CSHL was blessed by the generosity of the Montis in 2006, when we formed an alliance to fund cancer research and established the Tita Monti Cancer Research Laboratory, with the goal of funding better ways to help patients fight cancer.

In 2007, Joseph Monti died at the age of 88, after successful careers in the entertainment, restaurant, hospitality, and real estate industries. But he was admired for so much more, including devotion to his family, friends, and community. While most influential, he was a very gentle man who was loved by all whom he touched. Mostly, he was a family man whose devotion to Tita, his children, and grandchildren was apparent to all. He brought this same devotion to the family's dream of alleviating the pain and suffering caused by cancer. We express our deep condolences to their daughter Caroline Monti Saladino who is the president of the Foundation, son Richard, and to their entire family and supporters. We appreciate their continued inspiration. Through research, we will prevail over diseases and other causes of human suffering.

See previous page for photos of the following scientific staff:

Row 1: O. Anczukow-Camarda, M. Aaboe Jensen (Krainer Lab); C. Bautista (Krainer Lab); S. Pai (Brody Lab); A. Vestin (Mills Lab)

Row 2: L. Hufnagel (Wigler Lab); G.H. Otazu Aldana (Zador Lab); N. Alston (Mills Lab); A.D. Haase (Hannon Lab)

Row 3: S. Locke, D. Irvine (Martienssen Lab); M. Rooks (Hannon Lab), G. Collins (Tansey Lab); D.C. Gao (Mittal Lab); L. Cardone, D. Rebolini (McCombie Lab)

Row 4: A. Girard (Hannon Lab); P. Bommert (Jackson Lab); M. Chen (Trotman Lab); F. Rollins (Hannon Lab)

Row 5: O. Fregoso (Krainer Lab); A. Garrick (Wigler Lab); J. Zuber (Lowe Lab); L. Schiapparelli (Cline Lab)

CANCER: GENE EXPRESSION AND PROLIFERATION

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

Bruce Stillman's lab studies the mechanism by which DNA is replicated within cells. 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 cycle when DNA synthesis occurs. Among these are proteins that facilitate the assembly of chromatin, the "spools" around which DNA wraps tightly for packing into the chromosomes. The lab has also focused on the means by which chromatin assumes structural configurations that serve to propagate gene-expression information across generations—an important aspect of epigenetics. The prime focus of current research is the mechanism that initiates DNA replication in eukaryotic cells. At its heart is a protein that binds to "start" sites on the chromosomes, called the origin recognition complex (ORC). Stillman has demonstrated that ORC is also involved in the inheritance of centrosomes, the spatial control of DNA replication in the nucleus, and the timing of chromosomal copying during S phase.

David L. Spector and colleagues study the spatial organization and regulation of gene expression. Their in vivo approach is exemplified in a live-cell gene expression system that has made possible examination in real time of the recruitment of members of the gene expression and silencing machineries. One of the lab's current research foci is the distribution of polycomb proteins within the cell nucleus, which are known to keep genes in a silent state. Spector's team seeks to target them to segments of DNA as a means of selectively silencing specific genes. The lab is also studying long noncoding RNA molecules retained in the cell nucleus. A small fraction of expressed RNAs actually encode for proteins. Many of the other RNAs that are expressed are thought to perform yet undiscovered regulatory roles. The lab is systematically screening and characterizing large, noncoding RNAs that sublocalize in the cell nucleus with the ultimate goal of elucidating new mechanisms of regulating gene expression.

Leemor Joshua-Tor and her team are studying cell regulatory mechanisms, including protein complexes involved in nucleic acid regulatory processes. They use X-ray crystallography to obtain three-dimensional structures of individual proteins and atomic-level views of their interactions with other molecules. Biochemistry and molecular biology tools enable them to study properties that can be correlated with protein structure and function. Joshua-Tor's work on the helicase enzyme, which acts to unwind DNA strands during the DNA self-replication process, reveals how single DNA strands pass through structures called hexamer channels, and how DNA-binding hairpin structures collectively form a spiral "staircase" that sequentially tracks the DNA backbone. The lab has also solved the structure of a full-length protein called Argonaute, which they identified as Slicer, an enzyme that cleaves mRNA and is central to the RNA interference machinery.

Understanding gene expression—the remarkable process by which genetic information encoded in DNA is transcribed into strands of RNA, edited, and subsequently translated into proteins—is among the fundamental pursuits of molecular biology. Adrian Krainer's lab focuses on the posttranscriptional phase of gene expression, in which precursor mRNA molecules are read by specialized protein and RNA components, called splicing factors, that direct a molecular complex called the splicesosome to edit the strand in extremely precise ways, preparatory to translation. The lab simultaneously probes mechanisms of RNA splicing and ways in which they go awry in disease and the means by which faulty splicing can be compensated for or corrected. They have obtained insights into the causation of certain cancers and a potentially fatal neuromuscular disease called spinal muscular atrophy (SMA). Recently, they have studied pathways by which SF2/ASF, a splicing factor discovered in the lab, can transform cells. In SMA, they focus on strategies to correct a flaw in the RNA-editing process called exon skipping that affects expression of the mutated *SMN2* gene.

Work in William Tansey's lab follows from the intriguing observation, made by Tansey years ago, that the destruction of transcription factors is tied to their ability to activate transcription. Transcription factors are proteins that come into play when genes are expressed, involved early in the process by which genes direct the cell to manufacture proteins. But why and how is this first step in the life of a protein functionally related to proteolysis, the very last step—the protein's destruction by a molecular complex called the proteosome? The key link, Tansey believes, is ubiquitin, a molecule with which proteins must be tagged before they can enter the proteosome for destruction. Using a protein called Myc to study what happens during ubiquitin-mediated proteolysis, the lab has found that it is targeted by ubiquitin in a manner connected to its function. Overexpression of Myc is known to promote cancer. By "tagging" it with ubiquitin for destruction, cells limit the chance that the potentially lethal protein will linger.

Cancer chemotherapy typically focuses on preventing metastatic spread, yet insights have been lacking on how dormant metastatic lesions, after they have colonized distant organs, grow into large lethal lesions. Vivek Mittal and colleagues are shedding new light on this progression to macrometastasis. They study angiogenesis in the tumor microenvironment, which is composed of both malignant and nonmalignant cells. Among the latter are bone-marrow-derived endothelial progenitor cells, or EPCs, which, Mittal has demonstrated, become involved selectively in tumor blood-vessel growth. Recently, Mittal's team has shown that progression from dormant to active metastasis is controlled by an angiogenic switch turned on by bone-marrow-derived EPCs. Targeting the bone marrow compartment that gives rise to these EPCs, perhaps in conjunction with chemotherapy, may prove to be an effective strategy in fighting cancers in patients diagnosed after metastatic colonization has occurred.

Michael Myers seeks to understand how protein complexes regulate cellular behavior. Inside the cell, the majority of proteins can be found in highly interactive networks. Their architecture and how they change in response to the cellular environment are critical for the normal physiological functioning of the cell. In fact, these networks are responsible for the robustness and adaptability of living cells. Perturbations result in pathological conditions such as cancer and neurological disorders. Myers is using high-throughput mass spectrometry to gain insights into protein interaction networks from a variety of normal and pathological conditions.

Arne Stenlund and colleagues study the DNA replication properties of papillomaviruses, a large family of viruses that induce cell proliferation at the site of infection. In most cases, the resulting tumors are benign, but certain types of human papillomaviruses (HPVs) give rise to tumors that progress toward malignancy, most notably cervical cancer. Stenlund's group has obtained a detailed understanding of processes required for initiation of DNA replication from the papillomaviruses, using this specific system to gain a general biochemical understanding that is applicable in other systems. At the same time, they pursue studies aimed at developing an effective small-molecule inhibitor of human papillomaviruses that might someday be used by women who do not receive preventive anti-HPV vaccine or who are already infected with HPV.

STRUCTURAL BIOLOGY OF NUCLEIC ACID REGULATORY PROCESSES

L. Joshua-Tor C. Aksoy, J. Angiolillo, E. Enemark, C. Faehnle, H. He, P.R. Kumar, T. Schalch, S. Smith, N. Tolia

We study the molecular basis of cell 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 individual proteins and their interactions with other molecules. Biochemistry and molecular biology allow us to study properties that can be correlated with protein structure and function. Our efforts largely center on protein machines involved in nucleic acid regulatory processes.

DNA Translocation in a Replicative Hexameric Helicase

E.J. Enemark

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

Papillomaviruses are tumor viruses that cause benign and cancerous lesions in their host. Replication of papillomaviral DNA within a host cell requires the viral E1 protein, a multifunctional protein. E1 initially participates in recognizing a specific replication origin DNA sequence as a dimer with E2, another viral protein. Subsequently, further E1 molecules are assembled at the replication origin until two hexamers are established. These hexamers are the active helicases that operate bidirectionally in the replication of the viral DNA. To unwind DNA, helicases must separate the two strands while moving along, or translocating, on the DNA. On the basis of structures of the DNA-binding domain of E1 bound to DNA that we determined a few years ago in collaboration with Arne Stenlund's lab here at CSHL, 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 and the hairpins of apo-type subunits occupy the bottom positions of the staircase. The hairpins of the ADP-type subunits are placed at intermediate positions.

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

Mechanisms of RNAi

N. Tolia, T. Schalch, C. Faehnle, C. Aksoy, S. Smith
[in collaboration with G.J. Hannon and R.A. Martienssen, Cold Spring Harbor Laboratory; C. Mello, University of Massachusetts; J. Partridge, St. Jude Children's Resesarch Hospital]

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

During RNAi, long double-stranded RNA is processed to yield short (~19–31 nucleotides) double-stranded RNAs that trigger the RNAi response. These short RNAs get incorporated into effector complexes called the RNA-induced silencing complex (RISC), where in the mature complexes a single-stranded RNA, the antisense strand of the original double-stranded RNA, is retained in the complex. This short interfering RNA (siRNAs or microRNAs) then acts to guide the RISC complex to its target through base complementarity. The best-characterized pathway, and the one that is predominantly used for gene knockdown technology, is a posttranscriptional gene silencing (PTGS) pathway called "slicing." Here, the RISC complex is targeted to the mRNA and produces an endonucleolytic cut in the mRNA target, thus preventing gene expression from proceeding. Other RNAi silencing pathways such as translational inhibition and transcriptional gene silencing (TGS) are also mediated through RISC complexes. In all cases, these complexes contain a small single-stranded RNA and an Argonaute protein, features that serve to define the RISC complex.

In solving the structure of a full-length Argonaute protein a couple of years ago, we showed that a characteristic domain of these proteins, called the PIWI domain, belongs to the RNase H family of proteins. This finding was consistent with what was known about the biochemistry of the "slicing" reaction (the endonucleolytic cleavage of the mRNA by the RISC complex guided by the siRNA). Mutating the key aspartates identified at the active site to alanine in human Argonaute 2 abolished slicing. We could also show that a large positively charged groove along the protein could accommodate the siRNA and target (mRNA) binding, with the scissile phosphate at the active site. This was done by placing the 3′ end of the siRNA in a cleft of another characteristic domain, called the PAZ domain, in accordance to our previous results about 3′-end recognition by the PAZ domain as well as results from other laboratories.

Since then, we were able to produce recombinant human Argonaute 2, the only Argonaute in human cells able to carry out slicing, in an organism that lacks RNAi, namely, *Escherichia coli*. Together with Greg Hannon's laboratory here at CSHL, we showed that all that is needed to carry out slicing is an active Argonaute protein and an siRNA. This also enabled us to specifically examine what features of the effector step of RNAi reside in Argonaute in combination with the siRNA and what features must come from other components of RISC or perhaps other steps in the pathway.

We have been examining several Argonaute family members and found that the sole Argonaute protein in *Schizosaccharomyces pombe* is an active slicer. Importantly, together with Rob Martienssen's group here at CSHL, we showed that slicing is required for TGS in *S. pombe*.

We were also able to better define the active site of the Argonautes, as there are some unique aspects different from those of other RNase H enzymes. We have identified a third clade of the Argonaute family that is different from the Argonaute Argonautes and the Piwi Argonautes, which we named the Group-3 Argonautes. These appear to be specific to worms—*Caenorhabditis elegans* and *C. briggsae*—and consist predominantly of nonslicing Argonautes, meaning that they do not have an intact catalytic motif. Together with Craig Mello, we have shown that a group of secondary Argonaute proteins in *C. elegans* which act in a separate downstream silencing step belong to the nonslicing Argonautes of this third clade. These act following the initial trigger step that does require slicing, carried out by the well-characterized primary Argonaute RDE-1 in the exo-RNAi pathway or following the primary Argonaute ERGO-1 in the endo-RNAi pathway in worms.

NADP Regulates the Yeast *GAL* Induction System

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

Transcriptional regulation of the galactose metabolizing genes in *Saccharomyces cerevisiae* depends on three core

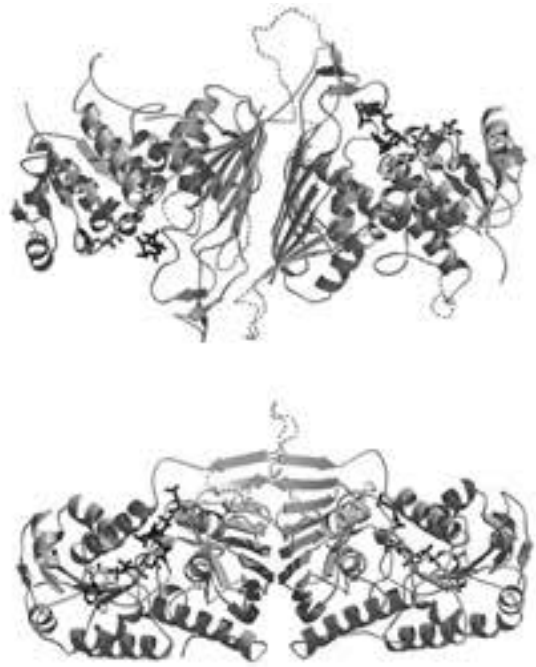


FIGURE 1 Two views of the Gal80p^{S0}-Gal4AD-NAD dimer. (*Gray ribbons*) Gal80p; the Gal4p-AD peptide and NAD in sticks. Disordered regions are shown as a dashed coil. The β-sheet regions of the carboxy-terminal domains form an extensive dimeric interface.

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

The crystal structures of Gal80p reveal a three-domain architecture with an amino-terminal domain consisting of a Rossmann fold, normally associated with binding of NAD(P) cofactors. The carboxy-terminal domain consists of a large β-sheet that forms an extensive dimer interface with another monomer (Fig 1). A large cleft is apparent between these two domains. A smaller third domain, located between the amino- and carboxy-terminal domains, consists of three small β-strands and a helix that resemble a set of fingers at the entrance of the cleft. Gal80p dimers form tetramers in both crystal forms. When we soaked a crystal of Gal80p^{S0}:P21 (a 21-amino-acid peptide that contains the conserved region of the carboxy-terminal activation domain of Gal4p) with the dinucleotide NAD, the structure revealed that NAD binds to both monomers of Gal80p in the cleft formed by the Rossmann fold. We were also able to observe extra density corresponding to the P21 peptide. The peptide appears to interact with the nicotinamide portion of the dinucleotide. NAD nestles between Gal80p and Gal4p, making several key interactions with Gal80p.

In vitro pull-down assays of Gal80p with purified recombinant GST-Gal4p containing the acidic activation domain (AD), in the presence of NAD and NADH showed no change in binding for either of these two dinucleotides. However, when NADP and NADPH were used, a clear reduction in binding is observed with increasing concentrations of NADP. Alterations in the NAD(P)-binding site affect the initial rate of *GAL* induction in vivo, but not overall final expression levels. It appears that NAD might facilitate Gal80p binding to Gal4p, since we could only identify Gal4p-AD with NAD bound, and NADP destabilizes this interaction. The mutations, affecting both NAD and NADP binding, would therefore disrupt both the stabilizing effect of NAD and destabilizing effect of NADP with a net result of faster induction for the mutants compared to wild type.

Although we do not understand precisely how this trigger for *GAL* regulation functions, nor the involvement of NADP versus NAD, we speculate that switching the cell to a fermentable galactose medium causes a change in NADP/NADPH or NADP/NAD ratios in the cell, and Gal80p effectively senses the metabolic state of the cell. NADP might be acting as a "second messenger" in triggering the system. Alternatively, Gal80p may function as an oxidoreductase enzyme, actively converting NADPH to NADP in the presence of a substrate causing it to disassociate from Gal4p.

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RNA SPLICING

A.R. Krainer G. Cutsail, O. Fregoso, M. Hastings, Y. Hua, M Jensen, R. Karni, Y. Liu, L. Manche, H. Okunola, X. Roca, S. Shaw, R. Sinha, S. Sun, M. Wallace, Z. Zhang

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, about 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. A summary of some of our recently published studies is provided below.

A SPLICING-FACTOR ONCOGENE

Alternative splicing has an important role in cancer, partly by modulating the expression of many oncogenes and tumor suppressors, and also because loss-of-function mutations that affect alternative splicing of various tumor suppressor genes account for some of the inherited and sporadic susceptibility to cancer. We tested the hypothesis that some of the factors that regulate alternative splicing events are causally involved in cancer; this work was done in collaboration with David Mu and Scott Lowe here at CSHL. We found that the splicing factor SF2/ASF and its antagonist hnRNP A1 are up-regulated in different sets of human tumors. In the case of SF2/ASF, this is sometimes due to increased copy number of its gene, *SFRS1*. *SFRS1* resides on chromosome 17q23, a region frequently amplified in breast cancer. Using retroviral transduction, we showed that slight overexpression of SF2/ASF is sufficient to transform immortal rodent fibroblasts, which form high-grade sarcomas in nude mice. The increased expression of SF2/ASF modulates the sensitivity to apoptotic stimuli and increases cell proliferation. In addition, SF2/ASF overexpression results in activation of downstream components of the PI3K/Akt/mTOR and Ras/MAPK signaling pathways, i.e., phosphorylation of S6K1 and eIF4E, respectively, bypassing upstream signaling. As expected from its biochemical activities, SF2/ASF overexpression affects alternative splicing of transcripts from many endogenous genes, including several tumor suppressors and oncogenes. One of the key targets is S6K1 pre-mRNA, resulting in increased expression of an unusual isoform of this kinase with oncogenic activity. shRNA-mediated down-regulation of either SF2/ASF or the novel S6K1 isoform is sufficient to reverse the transformed phenotype caused by SF2/ASF overexpression in vitro and in vivo, indicating that SF2/ASF has a role in tumor maintenance. These findings demonstrate that an alternative splicing factor, SF2/ASF, can act as an oncoprotein by modulating alternative splicing of critical target genes and is a potential target for cancer therapy.

ANTISENSE-CORRECTION OF SPLICING DEFECT IN SMA

Spinal muscular atrophy (SMA) is a neurodegenerative genetic disorder caused by the deletion or mutation of

the survival-of-motor-neuron gene, *SMN1*. An *SMN1* paralog, *SMN2*, differs 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. We continue to explore strategies to increase the extent of exon 7 inclusion during splicing of *SMN2* transcripts, for eventual therapeutic use in SMA. One of our strategies involves the use of antisense oligonucleotides (ASOs), in collaboration with Frank Bennett (Isis Pharmaceuticals). ASOs that target an exon or its flanking splice sites typically promote exon skipping. However, when we systematically tested a large number of ASOs with a 2′-*O*-methoxy-ethyl ribose (MOE) backbone that hybridize to different positions of *SMN2* exon 7, we identified several that promote greater exon inclusion, others that promote exon skipping, and still others that were neutral. This approach provides positional information about presumptive exonic elements or secondary structures with positive or negative effects on exon inclusion. The ASOs are effective not only in cell-free splicing assays, but also when transfected into cultured cells, where they affect splicing of endogenous SMN transcripts. The ASOs that promote exon 7 inclusion increase full-length SMN protein levels, demonstrating that they do not interfere with mRNA export or translation, despite hybridizing to an exon.

We then tested overlapping ASOs complementary to the flanking intronic regions, covering 60 nucleotides upstream or downstream from exon 7. As with the exonic ASOs, we classified them into positive, negative, and neutral with respect to their effects on exon 7 inclusion. Several of the positive ASOs were very potent when tested in cell-free splicing or in transfected cells, defining silencer regions in both flanking introns. We also tested them in transgenic mice that ubiquitously express human *SMN2* (Fig. 1). After intravenous administration, MOE ASOs are efficiently internalized by cells in peripheral tissues, and they are imported into the nucleus, where they can modulate gene expression. We observed efficient correction of the splicing defect in liver and kidney mRNA, and weaker correction in skeletal muscle. There was no correction in spinal cord—the tissue we need to target for SMA therapy—because the ASOs do not cross the blood-brain barrier. Therefore, in the future, we will explore methods for direct administration to the central nervous system.

The above experiments show that ASO tiling is a powerful method to map *cis*-acting elements that influence alternative splicing. To explore the mechanism of action of the silencer element in intron 7—which was first identified by Ravindra Singh and colleagues (University of Massachusetts Medical School, Worcester)—we introduced point mutations and assayed splicing of the resulting minigenes in transfected cells. Combined with splicing-factor overexpression and biochemical experiments, this analysis demonstrated that the intron 7 splicing silencer is recognized by the splicing repressor hnRNP A1 and other closely related members of the hnRNP A/B family.

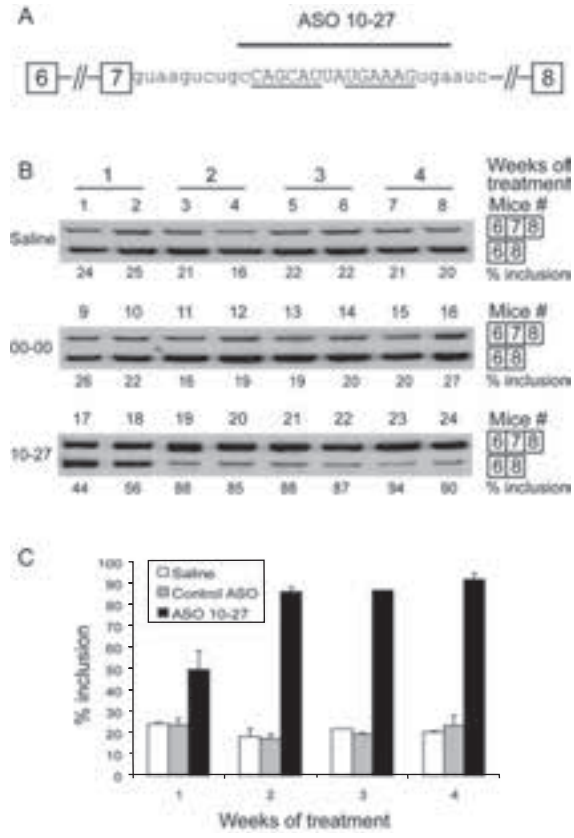

FIGURE 1 Correction of *SMN2* splicing defect by antisense oligonucleotide in transgenic mouse liver. (*A*) Diagram of the relevant portion of human *SMN2* pre-mRNA. The sequence at the 5′ end of intron 7 is shown. Two weak hnRNP A1 motifs that form an intronic splicing silencer are underlined. The position where a complementary antisense oligonucleotide (ASO 10–27) hybridizes to the pre-mRNA is shown by a horizontal bar. (*B*) MOE oligonucleotides were administered to *hSMN2* transgenic mice via the tail vein, twice a week, at 25 mg/kg. 00–00 is a random-sequence, negative-control oligonucleotide. Each oligonucleotide, or saline, was administered to eight mice, and the livers were harvested after 1, 2, 3, or 4 weeks of treatment (two mice each). Total RNA was extracted and radioactive reverse-transcriptase polymerase chain reaction (RT-PCR) carried out with a pair of *hSMN2*-specific primers. Radiolabeled PCR products were analyzed by native PAGE. The positions of the spliced mRNAs corresponding to exon 7 inclusion or skipping are indicated. (*C*) The gels shown in *B* were quantitated on a phosphorimager. The histogram shows the extent of exon 7 inclusion in response to each treatment.

CHARACTERIZATION OF SPLICING FACTORS

Splice sites with weak matches to the consensus sequences are common, although it is not clear how such sites are efficiently utilized. Using an in vitro splicing-complementation approach, we identified PUF60 as a factor that promotes splicing of an intron with a weak 3′ splice site. PUF60 has homology with U2AF^{65}, a general splicing factor that facilitates 3′ splice site recognition at the early stages of spliceosome assembly. We demonstrated that PUF60 can functionally substitute for U2AF^{65} in vitro, but splicing is strongly stimulated by the presence of both proteins. Reduction of either PUF60 or U2AF^{65} in cells by RNA interference alters the splicing pattern of certain endogenous transcripts, consistent with the idea that regulation of PUF60 and U2AF^{65} levels can dictate alternative splicing patterns. Thus, it appears that recognition of 3′ splice sites involves different U2AF-like molecules and that modulation of these general splicing factors can have profound effects on splicing.

We also characterized a protein phosphatase involved in pre-mRNA splicing. Several kinases and phosphatases participate in pre-mRNA splicing regulation, but their precise roles and the identities of their cofactors and substrates remain poorly understood. We showed previously that the human Ser/Thr phosphatase PP2Cγ promotes spliceosome assembly. We have now shown that PP2Cγ's distinctive acidic domain is essential for its activity in splicing and interacts with YB-1, a spliceosome-associated factor. Moreover, PP2Cγ is a phosphoprotein in vivo, and its acidic domain is phosphorylated under splicing conditions in vitro. PP2Cγ phosphorylation enhances its interaction with YB-1 and is reversed by the phosphatase in *cis*. PP2Cγ knockdown leaves constitutive splicing unaffected, but it inhibits cell proliferation and affects alternative splicing of CD44 pre-mRNA, a known YB-1 target. This effect on splicing regulation is mediated by PP2Cγ's acidic domain, which is essential to promote inclusion of CD44 exons v4 and v5 in vivo. We proposed that PP2Cγ modulates alternative splicing of specific pre-mRNAs coregulated by YB-1.

SR proteins are essential splicing factors with one or two RNA-recognition motifs (RRMs) and a carboxy-terminal Arg/Ser-rich (RS) domain. SR proteins bind to exonic splicing enhancers (ESEs) via their RRM(s) and are thought to promote splicing by antagonizing splicing silencers, recruiting other components of the splicing machinery through RS-RS domain interactions, and/or promoting RNA base pairing through their RS domains. We previously reported that the RS domain of the SR protein SF2/ASF is dispensable for in vitro splicing of some pre-mRNAs. In collaboration with Gourisankar Ghosh (University of California, San Diego), we have now extended these findings by identifying a short inhibitory domain in SF2/ASF; deletion of this amino-terminal segment permits splicing in the absence of this SR protein's RS domain of an IgM pre-mRNA substrate previously classified as RS-domain-dependent. Splicing of this pre-mRNA with SF2/ASF lacking its RS domain still requires an ESE, suggesting that an SR protein RS domain is not always required for ESE-dependent splicing activation. These data provide additional evidence that the SF2/ASF RS domain is not strictly required for constitutive splicing in vitro, contrary to prevailing models for how the domains of SR proteins function to promote splicing.

SPLICE-SITE RECOGNITION, GENOMICS, AND GENETICS

In collaboration with Michael Zhang's group here at CSHL, we characterized a novel class of splice sites called dual-specificity splice sites. These splice sites can be alternatively recognized as either 5′ or 3′ splice sites, and they match a composite of canonical 5′ and 3′ splice site consensus sequences, with a CAG|GURAG core. The relative use of a dual site as a 5′ or 3′ splice site can be accurately predicted by assuming competition for specific binding between spliceosomal components involved in recognition of 5′ and 3′ splice sites, respectively. Dual-specificity splice sites exist in humans and mice, and possibly in other vertebrate species, although most sites are not conserved, suggesting that their evolutionary origin is recent.

We continued to analyze the specificity of 5′ splice site recognition and the relationship between 5′ splice site mutations and genetic diseases. In many cases, mutations can disrupt splicing even though they do not disrupt the match to the splice site consensus. In collaboration with Ravi Sachidanandam here at CSHL, we used comparative genomics, i.e., large-scale data sets of splice sites from five different genomes, to identify pairwise dependencies between individual nucleotides of the 5′ splice site as a conserved feature of the entire set of 5′ splice sites. These dependencies are also conserved in human-mouse pairs of orthologous 5′ splice sites. Many disease-associated mutations disrupt these dependencies, as can some human single-nucleotide polymorphisms (SNPs) that appear to alter splicing, suggesting that 5′ splice site SNPs have a role in complex diseases.

We participated in other related studies at the interface among splicing, informatics, and human genetics.

A collaborative study of *NF1* splicing mutations from neurofibromatosis type-1 patients with Katharina Wimmer (Universitat Wien) helped to derive predictive rules for the outcome of mutations that disrupt 5′ splice sites. A database of aberrant 5′ splice sites in human disease genes was constructed and analyzed in collaboration with Igor Vorěchovský (University of Southampton School of Medicine) and Emanuele Buratti (ICGEB, Trieste). Another collaborative study with Dr. Brage Andresen (Aarhus University Hospital) showed that a missense mutation in exon 5 of the medium-chain acyl-CoA dehydrogenase (*MCAD*) gene that causes skipping of the exon in the context of MCAD deficiency by inactivating an ESE can be suppressed by an SNP that inactivates a nearby exonic splicing silencer. This finding illustrates that the phenotypic effects of splicing mutations need to be assessed in the context of the relevant haplotype.

REMODELING OF SPLICED mRNP BY eIF4A3

Pre-mRNA splicing not only removes introns and joins exons to generate spliced mRNA, but also results in remodeling of the spliced mRNP, influencing various downstream events, such as NMD (nonsense-mediated decay). This remodeling includes the loading of an exon–exon junction complex (EJC). To test whether EJC assembly or EJC components are required for pre-mRNA splicing, we immunodepleted the EJC core component eIF4A3 from HeLa cell nuclear extract and found that eIF4A3 is dispensable for pre-mRNA splicing in vitro. However, we found that eIF4A3 is required for the splicing-dependent loading of the Y14/Magoh heterodimer onto mRNA. Surprisingly, the loading of six other EJC components was not affected by eIF4A3 depletion, suggesting that their binding to mRNA involves different or redundant pathways. Finally, we found that the assembly of the EJC onto mRNA occurs at the late stages of the splicing reaction and requires the second-step splicing and mRNA-release factor HRH1/hPrp22, a DExD/H-box type RNA helicase and RNA-dependent ATPase. The EJC-dependent and -independent recruitment of RNA-binding proteins onto mRNA suggests a role for the EJC in mRNP remodeling involving interactions with other proteins already bound to the pre-mRNA, which has implications for NMD and other mRNA transactions.

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THE TUMOR MICROENVIRONMENT

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The microenvironment dictates the neoplastic potential of a growing tumor. For example, it influences the proliferation rate of the tumor, determines its metastatic potential and location of metastatic disease, and, most importantly, impacts the outcome of therapy. Therefore, its not surprising that several drugs targeting various components of the tumor microenvironment have successfully made their way into clinical trials.

For instance, Avastin, a humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF), has been approved by the FDA and used in the treatment of metastatic colorectal cancer in combination with standard chemotherapy. Similarly, other antiangiogenic drugs such as combretastatin, which is a vascular disruptive agent; VEGF-Trap-decoy receptors that are VEGF antagonists; and R1 and R2 antibodies that antagonize VEGF receptors are also in various phases of clinical trials. The kinase inhibitor Gleevac has also met FDA approval; it targets the pericyte component of the microenvironment via its ability to block the platelet-derived growth factor receptor (PDGF-R) kinase. Inhibitors targeting other components of the tumor microenvironment such as of fibroblasts and the extracellular matrix (ECM) are also in the pipeline.

The bone marrow (BM) contributes significantly to tumor progression. Although the BM-derived hematopoietic progenitor cells promote tumor angiogenesis via the release of proangiogenic factors or by creating permissive conditions in the tumor microenvironment that favor the growth of locally derived blood vessels, the endothelial progenitor cells (EPCs) are believed to provide an alternative source of endothelial cells that luminally incorporate into nascent blood vessels as bona fide endothelial cells. The BM also contributes in the initiation of metastasis. Malignant primary tumors bookmark sites in target organs by establishing viable premetastatic niches comprising BM-derived VEGF-R1$^+$ and CD11b$^+$ hematopoietic progenitor cells. These niches provide permissive local microenvironments for recruiting the incoming tumor cells, leading to the initiation and establishment of micrometastases. The existence of a BM reservoir of progenitor cells, and their selective involvement in tumor neovascularization, metastasis initiation, and progression has attracted considerable interest because these cells represent novel targets for therapeutic intervention.

In my laboratory, we are using a multidisciplinary approach to investigate the cellular and molecular mechanisms governing the contribution of hematopoietic stem cells (HSCs) and EPCs in tumor growth, metastasis initiation, and progression. In the last few years, we have systematically addressed these issues by using a powerful combination of mouse genetic models, functional genomics, and BM transplantation (BMT) approaches. Using this strategy, we demonstrated the biological role of EPCs in angiogenesis-mediated tumor growth and showed that ablation of one critical and specific component of the tumor microenvironment is a feasible approach for preventing tumor growth. We have extended our analysis of primary tumors toward elucidating the progression of metastatic lesions and have demonstrated that a second angiogenic switch underlies the progression of avascular lung micrometastases to vascular macrometastases. Importantly, ablation of EPCs did not affect initial colonization by metastatic cells but markedly impaired their progression into macroscopic lesions as a consequence of reduced neovascularization.

In the 2006 Annual Report, we had indicated our progress in unraveling hierarchies of the endothelial lineage in vivo, so that genome-wide analysis of gene expression, microRNA expression, and epigenetics could be performed. This analysis would determine how the niche microenvironment regulates the balance between cellular self-renewal and differentiation and, importantly, how tumors influence migration of EPCs out of the niche and how EPCs recruited to early tumors differentiate into mature endothelial cells and luminally incorporate into nascent neovessels. In this context, we have already optimized fluorescence-activated cell sorting (FACS) of specific populations, mRNA amplifications, and microarray hybridization.

Gene expression analysis has begun to reveal interesting themes. For example, we have observed that undifferentiated EPCs express more proangiogenic factors such as VEGF, PDGF, collagenase, and various

chemokines; however, once they differentiate and incorporate into vessels, these proangiogenic factors are down-regulated and negative regulators (TSP1, CD36, angiopoietin) of angiogenesis are up-regulated. In addition, the involvement of neuronal guidance pathways such as Netrin-Neogenin, EphrinB2-B4, Semaphorin-Plexin D1, and Robo-Slit that have a role in vascular patterning were also revealed in the gene expression profiling analysis.

To understand the roles of these potential candidate genes in vivo, we have investigated into EPC-specific promoters that could be used to express transgenes in EPCs in vivo. We have analyzed the promoters of these genes (Id1, VEGF-R2, and VE-cadherin) for their ability to mark EPCs in vivo. The proximal promoters were cloned into a lentivirus and used to drive two fluorescent markers (red and green fluorescent proteins). Of these, the Id1 promoter was found to specifically mark EPCs in the early BM, peripheral blood, and the tumor. We have investigated whether the exquisite property of the Id1 promoter to mark EPCs can be exploited for cell-specific ablation or generating loss of EPC function. The Id1 promoter was used to drive a suicide gene, herpes simplex virus thymidine kinase (HSV-TK). Administration of ganciclovir specifically ablated EPCs in TK BMT mice. EPC ablation impaired tumor growth associated with reduced vessel density. In another powerful approach, we have used RNA interference (RNAi) for suppressing the expression of EPC-specific genes (e.g., Id1 and VEGF-R2) in vivo. Suppression of Id1 or VEGF-R2 in EPCs resulted in mobilization defects and impaired angiogenesis-mediated growth of tumors. Collectively, our results suggest that these promoters are useful reagents to investigate EPC function in vivo.

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PROTEOMICS

M. Myers R. Bish
O. Fregoso
D. Perkowski

The completion of the human genome has ushered in a new age of biological discovery. This accomplishment has essentially identified all of the players governing human biology. The important work of assigning functions to this myriad of proteins has become one of the principal tasks of modern biology. Although many functions will be assigned using genetics or bioinformatics, the majority of this functional characterization will be performed by proteomics.

The goal of our laboratory is to understand how proteins and protein complexes regulate cellular behavior. In the environment of a cell, almost all of the proteins can be found in a highly interactive network. These networks govern the complex cellular responses to both normal and pathological stimuli. My laboratory is focused on understanding the global architecture of this network and how this network generates robustness (error tolerance) and adaptability to the system and how this network is altered to produce and survive complex diseases, such as cancer.

Optimization of Protein Identification

R. Bish, O. Fregoso, D. Perkowski

Protein identification is the major tool of proteomics. We have been optimizing mass spectrometry (MS) for protein identification. MS has many advantages over other techniques for protein identification, especially its sensitivity. However, the improved sensitivity comes at a price, as the sample preparation becomes increasingly important to ensure success. We have optimized a number of parameters to increase this success rate.

One of the key steps of optimization is to minimize the time and number of operations between the biological sample and the mass spectrometer. Therefore, we have developed a number of streamlined protocols for coupling common purification techniques, principally immunoprecipitation, to mass spectrometry (IP-MS). At the heart of these techniques is the ability to digest the sample directly from single-step affinity purifications. This provides a decrease in the number of processing steps and improves the recovery of the sample, as the resulting peptides are more easily recovered than most proteins. Immunoprecipitations are an ideal purification strategy, because intact antibodies are resistant to proteolysis. Using this protocol, we have been able to rapidly analyze complexes formed by a number of proteins involved in signal transduction, the DNA-damage response, and tumorigenesis. The increased sensitivity of this approach has also resulted in a large increase in the number of posttranslational modifications typically identified per experiment.

Although the direct trypsinization of single-step affinity purifications has many applications, it often cannot be used for characterizing complex posttranslational modifications that function in a scaffolding role. This is due to the "contamination" of other proteins containing the same/similar modifications. Therefore, we have developed a tandem affinity purification scheme that incorporates a denaturing step. The first nondenaturing step is used to isolate the protein complex, and the protein complex is eluted from the affinity resin using a strongly denaturing buffer. A second affinity step is then used to purify the target protein away from noncovalently bound proteins, as well as from proteins, which bind nonspecifically during the first affinity step. As in the single affinity step purification, the sample is recovered from the second affinity step using proteolysis. This denaturing tandem affinity purification (DTAP) has proven to be ideally suited to characterizing ubiquitination and other complex posttranslational modifications.

Identification of an Ubiquitin-binding Zinc Finger Involved in DNA-damage Repair

R. Bish, O. Fregoso

DNA damage elicits a signaling cascade that results in cell cycle arrest and recruitment of the DNA-repair machinery to sites of DNA damage. Importantly, polyubiquitin chains have an important role in this recruitment. In fact, *BRCA1*, a gene identified from hereditary forms of breast cancer, is a critical factor for DNA repair and it functions as an ubiquitin ligase. Interestingly,

BRCA1 elaborates a specific form of polyubiquitin, K6-linked chains. However, the substrates and effectors of this chain are unclear. Therefore, we used IP-MS to identify potential *BRCA1* effectors by identifying proteins that are associated with K6-linked polyubiquitin chains. This resulted in the identification of WHIP (Werner's helicase interacting protein). We have localized the ubiquitin-binding domain of WHIP to an amino-terminal zinc finger. Importantly, four other human proteins (polymerase κ, polymerase η, Rad18, and UBZ1) contain similar zinc finger domains, and we have demonstrated that all five domains bind polyubiquitin.

Similarly to most ubiquitin-binding proteins, WHIP is also ubiquitinated. Using DTAP, we have characterized the ubiquitination of WHIP and have found that it contains a mixture of K48-, K63-, and K11-linked ubiquitin chains, which is the first in vivo demonstration of this type of mixed ubiquitination. Interestingly, the ubiquitination of WHIP is responsive to DNA damage, as UV irradiation results in the rapid increase of WHIP polyubiquitination.

Importantly, the yeast homolog of WHIP, MGS1, has previously been implicated in having a role in DNA repair following UV damage. Using IP-MS, we have found WHIP to be in a larger network of DNA-repair proteins, including Werner's helicase, ERCC1, ERCC4, and the human RuvB homolog. Furthermore, WHIP-GFP (green fluorescent protein) fusion proteins appear to be localized to repair foci. Taken together, these data indicate that WHIP has a role in DNA-damage repair. Therefore, four of the five proteins containing ubiquitin-binding domains similar to those of WHIP are now known to be involved in DNA damage (WHIP, polymerase κ, polymerase η, and Rad18).

The restriction of a class of ubiquitin-binding domains to a single cellular task is highly unusual. Therefore, we are testing whether the remaining protein, UBZ1, also has a role in DNA damage. In fact, IP-MS data indicate that UBZ1 has a role in DNA-damage repair, as it is found in a complex with ubiquitinated proliferating cell nuclear antigen (PCNA), the complete Werner's helicase complex (WRN, Ku70, Ku80, and DNA-PK), and the complete Bloom's helicase complex (BLM, Topo 3a, and BLAP75). Importantly, UBZ1 also undergoes ubiquitination in response to UV irradiation and is recruited to repair foci. The ubiquitination of UBZ1 is also regulated by UV irradiation, but unlike WHIP, the polyubiquitination of UBZ1 decreases following UV irradiation. These data indicate that, like WHIP, UBZ1 has a role in DNA-damage repair. The molecular role of WHIP and UBZ1 in DNA-damage repair is currently being explored.

Genomic Tagging of Mammalian Proteins in Somatic Cells

O. Fregoso

Both the single-affinity purification and denaturing tandem-affinity purifications are highly flexible and can be adapted to overexpressed and native proteins. However, we have found that the use of epitope tags (FLAG, HA, MAT, etc.) produce higher-quality mass spectra, largely due to the uniformity and reproducibility of the affinity resins. Unfortunately, in mammalian cells, this usually requires the overexpression of the target protein, which can lead to any number of artifacts. Genomic tagging of proteins has proven to be a powerful tool in characterizing protein complexes in yeast. However, this type of genomic tagging has proven to be extremely difficult in mammalian somatic cells.

Using adeno-associated virus (AAV), we have been developing a strategy to epitope-tag proteins in the mammalian genome. AAV can be used to direct the site-specific integration of the epitope tag into the genome. The targeting requires about 1 kb of flanking homologous sequence. This tagging strategy relies on infections at high multiplicity, and many nonhomologous integrants are expected to occur, which will be difficult to select against using current selection schemes. Therefore, we have developed a novel selectable marker for use in epitope tagging that uses an intein to couple the marker to the translation, rather than the transcription, of the targeted protein. This results in at least a sixfold enrichment of targeted alleles over transcription-coupled (IRES)-based strategies. In fact, transfection with a GFP-intein-puro construct, followed by puromycin selection, results in more than 97% GFP-positive cells. We are currently engineering AAV to target members of the PARP (poly[ADP-ribose] polymerase) gene family.

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CELL BIOLOGY OF THE NUCLEUS

D.L. Spector Y.-C.M. Chen, Y. Fang, S. Hearn, M. Hübner, R.I. Kumaran, J. Li, T. Nakamura, H. Sunwoo, R. Thakar, J. Wilusz, M. Yuan, R. Zhao

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

The Dynamics of Gene Expression/Repression

M. Hübner, R.I. Kumaran, J. Li, T. Nakamura, R. Thakar, M. Yuan, R. Zhao

Much of our efforts during the past year relate to characterizing the spatial and temporal aspects of gene expression/repression. Our studies have utilized a cell line (U2OS 2-6-3), previously developed in our laboratory, that contains a transgene stably integrated as a 200-copy array in human chromosome 1p36 (Janicki et al., *Cell 116:* 683 [2004]). This cell system allows us to directly visualize gene expression (DNA, RNA, protein) within the context of a living cell. During the past year, we have developed additional lines that also stably express other proteins of interest, for example, the large subunit of RNA polymerase II (RNA pol II LS) fused to enhanced yellow fluorescent protein (EYFP) and lamin B1 fused to mCherry (a mutant red fluorescent protein). The stable expression of the aforementioned proteins allows us to study the dynamics of gene expression during the cell cycle. Using four-dimensional live-cell microscopy, we have analyzed the specific timing of the dissociation/association of the transcription machinery with a specific genetic locus upon entry into and exit from mitosis. We have found that RNA pol II LS starts to leave the locus between 0 and 6 minutes before nuclear lamina disassembly. At the end of mitosis, RNA pol II enters daughter nuclei between 2 and 6 minutes after nuclear lamina reassembly. However, RNA pol II LS is recruited to the genetic loci within the daughter nuclei between 0 and 8 minutes postnuclear entry. Studies are currently under way to determine whether other members of the gene expression machinery (i.e., capping factors, pre-mRNA slicing factors, and polyadenylation factors) are recruited to the locus in daughter nuclei concomitantly with RNA pol II or if they exhibit an ordered recruitment of individual proteins or subcomplexes.

To address the effect of nuclear position on gene activation, we developed an inducible live-cell system in which the position of the genetic locus can be targeted from a more internal nuclear region to the nuclear periphery in mammalian cells. Targeting was achieved by the expression of a lamin B1 fusion protein. Interestingly, the mechanism of targeting of the locus to the nuclear periphery required one round of cell division and nuclear reassembly. Once targeted, the locus remained anchored to the nuclear periphery in interphase as well as in daughter cells following passage through a subsequent mitosis. Upon transcriptional induction, components of the gene expression machinery were recruited to the targeted locus, and nascent transcripts were visualized at the nuclear periphery. The kinetics of transcriptional induction at the nuclear lamina was similar to that observed at an internal nuclear region. This new cell system is a powerful tool to study the dynamics of gene function at the nuclear periphery during normal physiology and in disease states, such as envelopathies, in living cells.

In addition to examining the role of the nuclear periphery in gene activation/silencing, we have also been interested in the silencing of endogenous genes by polycomb (PcG) proteins. Polycomb proteins are associated with regions of facultative heterochromatin, and members of the PRC1 or maintenance complex are present in PcG bodies. These bodies colocalize with chromatin enriched in histone H3 di- and trimethylated at lysine 27 (H3K27). We were interested in determining whether the induction of this histone mark at a specific chromosomal site triggers the de novo formation of

a PcG body at that site. To induce H3K27 methylation at the reporter locus, we targeted the PRC2 complex member EZH2, which has previously been shown to possess histone methyl transferase activity toward H3K27, to the locus. We were able to show that this fusion protein leads to efficient H3K27 trimethylation at the locus. Importantly, this resulted in the colocalization with the locus of the YFP-tagged PcG body marker protein Bmi1. A catalytically inactive EZH2 protein lacking the SET domain, however, does not lead either to H3K27 trimethylation or to PcG body formation at the locus. We next investigated whether the PcG body persists at the locus after removal of EZH2. To this end, we used an EZH2 fusion protein containing the tetracycline-inducible *trans*-activator (tTA)-binding protein. This fusion protein allows us to remove EZH2 from the locus within 2 minutes of doxycycline administration. We found that the PcG body persists at the locus in the absence of EZH2 for at least 8 hours.

We are currently investigating if the histone mark and PcG body are inherited to the daughter cells during cell division. We also wanted to determine if the newly formed PcG body has an inhibitory effect on the transcriptional inducibility of the locus. To this end, we quantified in single cells the MS2-YFP signal at the locus after induction with doxycycline. We found that the tethering of EZH2 to the locus significantly inhibited the transcriptional inducibility of the locus. In conclusion, we have developed several in vivo approaches to examine the dynamics of gene expression/silencing in living cells. Ongoing studies will elucidate the underlying principles of these dynamics and how they can be manipulated in vivo to regulate gene expression.

Identification and Characterization of Nuclear Retained Noncoding RNAs

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Although it has been generally assumed that most genetic information is expressed as and transacted by proteins, recent evidence from genomic tiling arrays and large-scale cDNA cloning projects suggests that the majority of the transcriptional output of the mammalian genome represents RNA that does not code for proteins. These noncoding RNAs include microRNAs, Piwi-interacting RNAs (piRNAs), and small nucleolar RNAs (snoRNAs), as well as a significant number of longer transcripts, most of whose functions are unknown. These longer transcripts probably do not simply represent transcriptional "noise" because many have been shown to exhibit cell-type-specific expression, localization to specific subcellular compartments, and association with human diseases. Therefore, the big and largely unanswered question that we are trying to address is: What are the functions of these long noncoding RNAs?

In collaboration with J. Mattick's laboratory (University of Queensland, Brisbane, Australia) we have examined noncoding RNAs (ncRNAs) whose expression levels change after mouse C2C12 myoblasts differentiate into myotubes. RNA FISH (fluorescence in situ hybridization) analysis of candidate clones revealed one clone that showed an exclusive nuclear retention of the ncRNA in a punctate distribution pattern. This pattern corresponded to paraspeckles, a recently identified nuclear domain whose function is unknown. Clone 22 encodes a 3.2-kb poly(A)$^+$ RNA transcript that is broadly expressed in adult mouse tissues and conserved among mammals. Microarray data indicated that this RNA is 4.6-fold more highly expressed in myotubes than in myoblasts, suggesting its potential function during differentiation. Interestingly, the same locus produces a longer 19-kb ncRNA by alternative 3′-end formation in addition to the 3.2-kb transcript. This longer ncRNA is also retained in the nucleus and localized to paraspeckles where it is indistinguishable from Clone 22. Ongoing studies are examining the mechanism by which these ncRNAs are retained in the nucleus and their potential function in myoblast differentiation.

MALAT1 is a long ncRNA that was originally identified as a transcript whose expression predicted metastasis of non-small-cell lung tumors and was subsequently shown to be overexpressed in many human cancers (for review, see Prasanth and Spector 2007). We have identified a highly conserved small (~60 nucleotide) ncRNA that originates from the MALAT1 locus and is broadly expressed in normal human tissues. In contrast to the stable long MALAT1 transcript that localizes to nuclear speckles, the approximately 60-nucleotide RNA exclusively localizes to the cytoplasm and has a short half-life (and thus we have named it mascRNA, MALAT1-associated small cytoplasmic RNA). mascRNA appears to represent a novel class of small RNAs based on its size and because it does not function as a microRNA precursor.

We propose that 3′-end processing of MALAT1 via a previously uncharacterized mechanism generates the mascRNA transcript. MALAT1 can generate two long transcripts (~6.7 kb and ~7 kb in mouse) that differ in the location of their 3′ ends. Cleavage/polyadenylation can occur to yield the longer approximately 7-kb MALAT1 isoform, but this isoform is expressed at a

very low level in cells. Instead, MALAT1 3′-end formation almost always occurs several hundred nucleotides upstream of the polyadenylation signal (to yield an ~6.7-kb isoform in mouse) via a mechanism that is in stark contrast to classic cleavage/polyadenylation. A single cleavage event downstream from a genomically encoded poly(A)-rich tract defines the 3′ end of the abundant approximately 6.7-kb MALAT1 transcript and the 5′ end of the mascRNA transcript. This mechanism allows a short poly(A) tail-like moiety to be present on the 3′ end of the abundant MALAT1 transcript. In support of this model, antisense oligonucleotides complementary to the mascRNA region inhibited MALAT1 upstream 3′-end processing (resulting in an accumulation of the ~7-kb transcript) and reduced mascRNA expression. Our findings reveal a novel 3′-end processing mechanism by which a single locus can yield both a stable nuclear retained ncRNA and a small cytoplasmic ncRNA.

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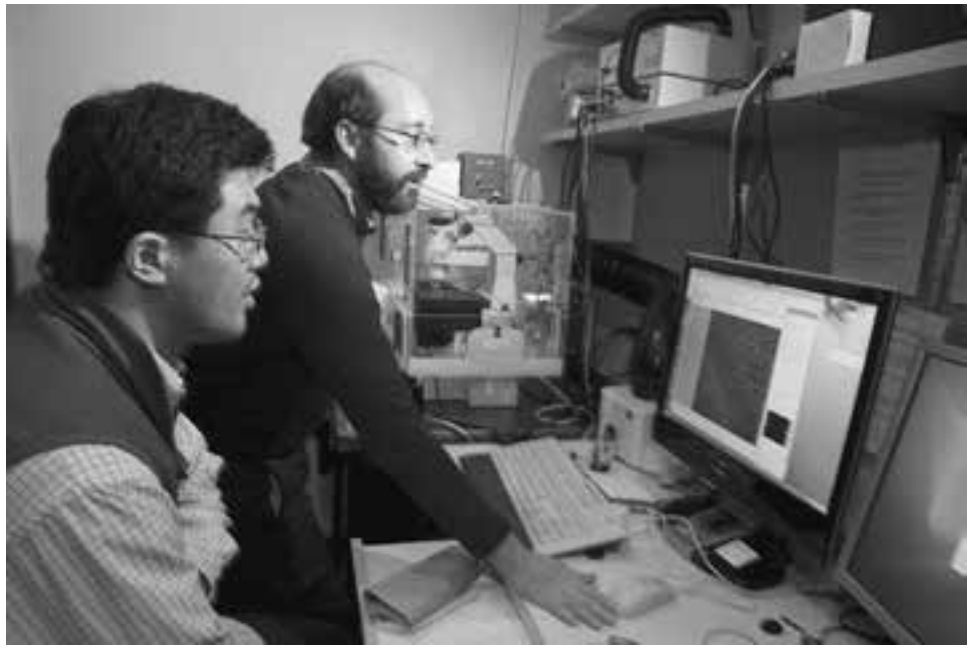

Hongjae Sunwoo and Stephen Hearn

MOLECULAR BIOLOGY OF PAPILLOMAVIRUSES

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S. Schuck

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

An impediment to the study of papillomaviruses has been the inability to define simple in vitro cell culture systems for analysis of the viral life cycle. These viruses normally require specialized differentiating cells that only with difficulty can be generated in cell culture. However, for bovine papillomavirus (BPV-1), a convenient cell culture system exists where viral gene expression, oncogenic transformation, and viral DNA replication can be studied. Thus, BPV has become a useful model for these aspects of the viral life cycle.

The DNA replication properties of the papillomaviruses show some unique and interesting characteristics. As part of their normal life cycle, these viruses can exist in a state of latency, which is characterized by maintenance of the viral DNA as a multicopy plasmid in infected cells. The copy number of the viral DNA is tightly controlled and the viral DNA is stably inherited under these conditions. Papillomaviruses therefore provide a unique opportunity to study plasmid replication in mammalian cells. In addition, the viral DNA replication machinery represents one of the most promising targets for antiviral therapy.

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

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

ADJACENT RESIDUES IN THE CONSERVED E1 INITIATOR β-HAIRPIN DEFINE DIFFERENT ROLES OF THE β-HAIRPIN IN LOCAL ORI MELTING, HELICASE LOADING, AND DNA HELICASE ACTIVITY

The mechanism through which local ori melting is achieved is not understood for any replicon. As reported previously, we have recently identified a specific form of the papillomavirus E1 initiator protein that is associated with local ori melting. This form, a double trimer (DT), is a required precursor for the double hexameric (DH) helicase and forms in the presence of ATP. On the basis of mutational analysis of the helicase domain of the E1 protein, a specific structural element, a β-hairpin, is specifically required both for the formation of the DT and for template melting. Mutation of H507 at the tip of the β-hairpin to alanine results in loss of both DT formation and ori melting.

To determine the specific role of this residue, we substituted histidine with a wide range of residues, including A, V, L, R, N, M, F, and Y and tested these mutant E1 proteins for DNA replication in vivo and in vitro. In vivo DNA replication assays demonstrated that only H507F had significant replication activity. DNA

replication assays in vitro, however, showed that both H507F and H507Y had activity similar to that of the wild-type protein, whereas the rest of the substitutions lacked activity.

To determine at which step in initiation of DNA replication the H507 has an essential role, we tested the H507 substitutions in a variety of assays that we have developed. By the electrophoretic mobility-shift assay (EMSA), we determined that the wild-type E1, H507F, and H507Y could form the DT complex. However, only the wild-type E1 and H507F could form the double hexameric (DH) helicase. Because of the location of the β-hairpin on the inside surface of the hexameric ring, we also tested all of the mutants for DNA helicase activity. Although we could observe slight (about twofold) differences in helicase activity of some H507 substitutions compared to wild-type E1, a helicase defect is not responsible for the replication defect of these substitutions because H507F, which is the only mutant with wild-type replication activity, had among the lowest activities. In contrast, mutation of the neighboring residue, K506, resulted in complete loss of DNA helicase activity, demonstrating that this particular residue is required for the helicase.

These results provide a clear demonstration that the β-hairpin is directly involved in both local ori melting and helicase activity of E1 and that mutation of neighboring residues distinguishes between these two activities. H507 is required specifically for local ori melting, whereas the adjacent residue, K506, is required for both local melting and DNA helicase activity. Consistent with these results, an alignment of known SF3 helicases demonstrates that K506 is well-conserved in both papovavirus and parvovirus, in agreement with the importance of this residue for the helicase activity of these proteins. In contrast, H507 is only conserved in the papovavirus group. This distinction between the papovavirus and parvovirus groups is consistent with the fact that papovaviruses have double-stranded DNA (dsDNA) genomes, whereas the parvoviruses have single-stranded DNA (ssDNA) genomes and therefore do not require a local ori melting activity.

These results now provide an explanation for how the local melting activity and the helicase activity are linked. In the DT, which initially melts the dsDNA, the β-hairpin, and specifically H507, is involved in melting the dsDNA, most likely through a direct interaction with the DNA. As the DNA is melted, additional E1 molecules are recruited to the complex, and as the helicase is formed, K506 in the β-hairpin now contacts the ssDNA in the helicase. This scheme provides an explanation for the transition between the local ori melting activity and the helicase activity and provides a novel mode of helicase loading in that the E1 helicase, in essence, loads itself through the DT precursor, which melts the DNA template.

ATP-DEPENDENT MINOR GROOVE RECOGNITION OF T-A BASE PAIRS IS REQUIRED FOR TEMPLATE MELTING BY THE E1 INITIATOR PROTEIN

Although local ori melting is an essential process in all organisms, surprisingly, little information is available about the process and the types of activities that perform the melting. For many years, it has been known that in prokaryotes, the protein DnaA is responsible for ori melting, although the mechanism is not understood. In eukaryotes, no activity has been identified that melts the ori in preparation for DNA replication. Viral initiator proteins such as the E1 protein from papillomaviruses have long been known to be responsible for local ori melting, as detected by oxidation of un-base-paired T residues in the ori, although the mechanism is not known. To define the local ori melting process, we have examined this process in detail, including the sequence dependence for local melting. We have determined that local melting is not a single event but consists of several events that happen in sequence. All of these events are carried out by a double trimer (DT) of E1.

The helicase domain in the E1 protein specifically recognizes a stretch of six T-A base pairs that flank the E1-binding site. This recognition occurs in the minor groove and results in local melting of the six T-A base pairs. As expected from this result, the six T-A base pairs are also essential for formation of the double hexamer (DH), for unwinding of the ori, and for DNA replication in vivo. In addition to melting of the six T-A base pairs, which likely results from a direct interaction between the E1 β-hairpin and the six T-A base pairs, the six T-A base pairs also direct melting 5–6 base pairs away from the T-A base pairs, toward the E1-binding site. This melting is likely generated by interaction of the oligomerization domain in E1 and the DNA (Fig. 1). These events account for melting of about 15 base pairs on each side of the E1-binding site. The E1-binding site is clearly melted by a different mechanism, most likely by the untwisting of the DNA by the two trimers. Together, these data suggest a simple model for how E1 may be able to melt large regions of DNA using simple means. One consequence of the dual interactions of each E1 molecule with DNA is that each interaction need only be responsible for melting of a few base pairs because a total of 6 E1 molecules and 12 interactions are involved in melting in the E1 DT.

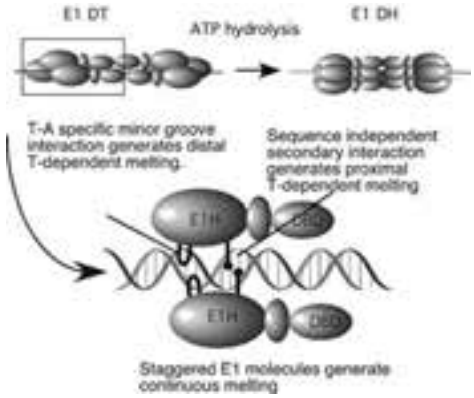

FIGURE 1 Model for how multiple E1 DNA interactions generate large-scale melting. The E1 molecules in the DT bind in a helical arrangement, wrapping around the DNA duplex. Each E1 molecule contacts DNA in two positions, separated by one-half turn of the helix. These contacts correspond to distal and proximal T-dependent melting, respectively. Together, these six contacts account for the approximately 15–18 base pairs of permanganate reactivity observed in the left half of the ori. For clarity, only two of the three staggered E1 molecules are shown.

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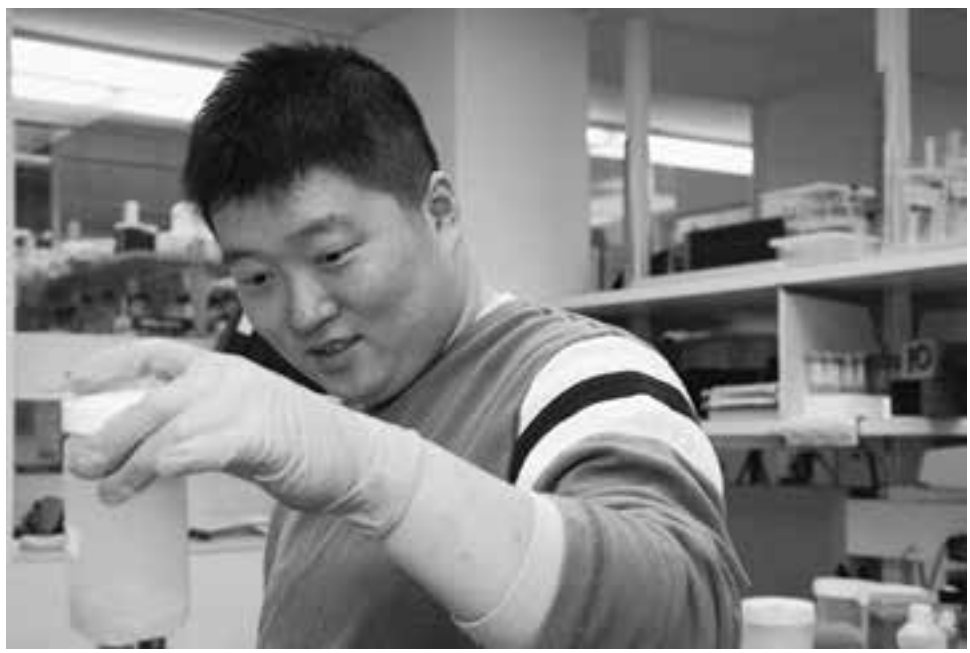

Xiaofei Liu

DNA REPLICATION AND CHROMATIN INHERITANCE

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Research continues to focus on the mechanism of initiation of DNA replication in cells from eukaryotes and how specific states of gene expression are inherited from one cell generation to the next. In rapidly proliferating cells, such as cancer cells, the process of duplicating the cellular chromosomes begins soon after the chromosomes are separated during mitosis. Previous research has demonstrated that four proteins, the origin recognition complex (ORC), Cdc6, Cdt1, and the minichromosome maintenance (MCM) proteins, are required for establishing a pre-replicative complex (pre-RC) at sites along the chromosomes, many of which are destined to become origins of DNA replication in the S phase of the cell division cycle, the phase when DNA synthesis occurs.

DEFINING ORIGINS OF DNA REPLICATION

During the last few years, much of our focus has been on investigating the detailed interaction between ORC and Cdc6, the very first step in the process of initiating DNA replication. Although ORC binds to many sites in the yeast genome, it does not solely determine the location of origins because many ORC-binding sites are not origins and ORC does not bind to all predicted origin DNA sequences. Studies in past years have shown that Cdc6 contributes to origin specificity and later cooperates with ORC and Cdt1 to load the MCM proteins onto the DNA, thereby creating competent pre-RCs for subsequent initiation. ORC and Cdc6 combine to form a ring-shaped complex that contains six AAA+ subunits. ORC and Cdc6 ATPase mutants are defective in MCM loading and ORC ATPase mutants have reduced activity in ORC–Cdc6–DNA complex formation.

Last year, the role of the Cdc6 ATPase on ORC–Cdc6 complex stability was examined in the presence or absence of specific DNA sequences. The Cdc6 ATPase was activated by ORC; it regulated ORC–Cdc6 complex stability, and Cdc6 ATPase activity was suppressed by certain DNA sequences with the functional origin DNA. Mutations in the conserved origin sequences that are required for initiation of DNA replication induce Cdc6 ATPase activity and prevent stable ORC–Cdc6 association with the DNA. By analyzing ORC–Cdc6 complex stability on various DNAs, it was demonstrated that specific DNA sequences control the rate of Cdc6 ATPase, which in turn controls the rate of Cdc6 dissociation from the ORC–Cdc6–DNA complex.

On the basis of these and previous data, a mechanism was proposed explaining how Cdc6 ATPase activity promotes origin DNA sequence specificity: On DNA that lacks origin activity, Cdc6 ATPase promotes dissociation of Cdc6 from the ORC bound to the DNA. Furthermore, origin DNA down-regulates Cdc6 ATPase activity, resulting in a stable ORC–Cdc6–DNA complex, which can then promote MCM loading, a process that requires Cdc6 ATPase activity. This model for Cdc6 ATPase having a dual role in determining origin location in the DNA has obvious relevance for origin specificity in higher eukaryotes.

THE STRUCTURE OF ORC

To determine in detail how the process of assembly of the DNA replication apparatus occurs on chromosomes, we began structural studies on ORC and the ORC–Cdc6 complex. Previous studies using single-particle reconstruction of ORC from electron micrographs, in collaboration with Huilin Li's group at Brookhaven National Laboratory (BNL), have shown that ORC consists of a multi-lobed complex that binds Cdc6 to form a ring-shaped structure. In the past year, we devised a molecular tagging procedure that attaches the *Escherichia coli* maltose-binding protein (MBP) to either the amino or carboxyl terminus of each of the five largest ORC subunits and purified all ten possible ORC complexes. Electron microscopy of the purified ORC–MBP complexes and two-dimential projections of the ORC–MBP particles allowed the location of the MBP density to be determined and hence the location of the termini of each of the five largest ORC subunits within the ORC structure. Consistent with the studies on reconstitution of the human ORC, the structure of yeast ORC showed that the Orc2 and Orc3 subunits were adjacent to each other and formed the base of the ORC structure. The Orc4 and Orc5 subunits also formed a subdomain in the middle of

ORC. The Orc1 subunit was located adjacent to the Orc4 and Orc5 subunits and seemed to form a lobe on the top of ORC. The location of Orc6, the only subunit not tagged with MBP, was determined by comparing the structures of ORC containing all six subunits with a structure of the Orc1,2,3,4,5 complex lacking Orc6. Orc6 localized adjacent to the Orc2,3 subdomain at the base of ORC. This structural information is being used to identify how ORC associates with DNA, how ATP controls the function of ORC, and how ORC interacts with DNA and other DNA replication proteins.

DYNAMICS OF ORIGIN RECOGNITION IN HUMAN CELLS

Unlike in yeast cells, where ORC is a stable complex throughout the cell division cycle and appears to be bound to origins at all times, the dynamics of ORC throughout the human cell division cycle is very different. ORC is composed of six subunits and in contrast to other eukaryotic initiators examined thus far, ORC in mammalian cells is assembled and disassembled in a cell-cycle-dependent manner. Assembly of human ORC in vitro was found to be dependent on ATP binding to the Orc4 and Orc5 subunits, because mutations in the ATP-binding sites of Orc4 or Orc5 impaired complex assembly. In contrast, Orc1 ATP-binding was not required for ORC assembly. Immunofluorescence staining of human cells with anti-Orc3 antibodies demonstrated cell-cycle-dependent association with a nuclear structure. Immunoprecipitation experiments showed that ORC disassembled as cells progressed through S phase. ORC then reassembled during late mitosis and early G_1 phase. These data suggested that the assembly and disassembly of ORC in human cells are uniquely regulated and may contribute to restricting DNA replication to once in every cell division cycle.

The largest subunit of ORC, Orc1, was shown to be degraded during the G_1- to S-phase transition. To follow up on this observation, the dynamics of Orc1 during the cell division cycle was investigated. Time-lapse live-cell images of human cells expressing fluorescently tagged-Orc1 revealed a dynamic nuclear localization pattern during G_1 phase. Later during S phase, the MCM helicase proteins and proliferating cell nuclear antigen (PCNA), a DNA replication fork protein, displayed sequential waves of spatiotemporal patterns similar to those of Orc1 in G_1. In vivo labeling studies revealed spatial overlap between the Orc1 distribution in G_1 and DNA replication patterning from cells pulse-labeled with bromodeoxuridine or fluorescent dNTP during the previous S phase.

These results suggest that the dynamic distribution of Orc1 during G_1 phase might pattern the nucleus for subsequent events in S phase that lead to both spatial and temporal control of DNA replication. In mammalian cells, chromosomal domains occupy a specific nuclear position and replicate at defined times during the S phase of the cell division cycle. Thus, the spatiotemporal pattern of DNA replication within a given cell type is heritable and is linked to developmentally regulated gene expression. The studies on Orc1 dynamics may have uncovered a fundamental link between the organization of DNA replication and events that determine when loci are activated during cell lineage specification.

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

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Ubiquitin (Ub)-mediated proteolysis is a process in which covalent attachment of Ub to target proteins signals their destruction by the 26*S* proteasome. The controlled destruction of proteins by this system is used to fine-tune the levels of proteins within the cell, allows cells to rapidly alter their protein composition in response to particular signals, and provides rigorous control and directionality to processes such as the cell cycle. Our particular interest centers on how Ub-mediated proteolysis regulates gene transcription. In recent years, a body of evidence has accumulated suggesting that the Ub–proteasome system (UPS) is directly involved in the regulation of gene activity. We are interested in understanding how the UPS regulates transcription and the consequences of this regulation for the control of cell growth in normal and cancer cells.

Research in our laboratory is divided in two areas. To study the basic processes that connect the transcription and Ub systems, we perform biochemical and genetic experiments using the baker's yeast *Saccharomyces cerevisiae* as a model system. To understand the biological significance of the intersections between these systems, we study the human oncoprotein transcription factor Myc.

REGULATION OF MYC

Myc is a basic helix-loop-helix leucine-zipper transcription factor that features prominently in the control of cell growth. Capable of acting as both a transcriptional activator and repressor, Myc controls the expression of genes required for cell growth and division. Consistent with the type of genes that it regulates, Myc is also is a major human oncoprotein that features in a variety of human cancers including leukemia, lymphoma, breast, colon, and lung cancer. It is estimated that altered Myc expression or activity contributes to about 70,000 cancer deaths in the United States each year—about one third of all cancer fatalities.

Like many transcription factors, particularly those involved in the control of cell growth, Myc is an unstable protein that is destroyed by Ub-mediated proteolysis. Previous work in our laboratory has revealed that the destruction of transcription factors such as Myc is frequently connected to their ability to activate transcription. Specifically, we have found that the same domain in these proteins that allows them to activate transcription is responsible for their destruction. We have also found that, in some cases, transcription factors *need* to engage the Ub system and become ubiquitylated in order to function. This intimate connection between transcription factor activity and turnover has two important ramifications. First, it predicts that components of the ubiquitin–proteasome system will be directly and intimately involved in the control of gene expression. Second, it reveals that if we study the destruction of transcription factors, we can learn not just about activator proteolysis, but also about activator function. Thus, we believe that by probing the mechanisms through which Myc is destroyed by the UPS—the elements involved, the cellular players that control Myc turnover, and the pathways that govern Myc stability—we can also gain new insight into how this enigmatic human oncoprotein functions.

Our current research is focused on exploiting the connection between activity and turnover to learn more about how Myc regulates gene expression programs relevant to oncogenic transformation. Much of our work is focused on the highly conserved elements within Myc known as "Myc boxes" (Mb). The first two Myc boxes—MbI and MbII—are within the transcriptional activation domain of Myc and have been extensively characterized for their contribution to Myc activity and instability. More recently, however, we have turned our attention to another highly conserved element within Myc, "MbIII," that we have found contributes to Myc destruction and also has a critical role in Myc's ability to regulate apoptosis and oncogenic transformation in vitro and in vivo. Although MbIII was first noted more than 20 years ago, it has not been studied extensively. Our finding that MbIII is an important part of the Myc protein has provided us with an opportunity to probe novel aspects of Myc function.

Focus on MbIII has centered on its contribution to Myc-driven target gene regulation. We have found that

MbIII is largely dispensable for the ability of Myc to activate gene activity but is important for its ability to repress Myc target genes. To understand the mechanism through which MbIII contributes to repression, we have used chromatin immunoprecipitation (ChIP) analysis to probe transcriptionally relevant events that are modulated by Myc in an MbIII-dependent manner. This analysis has revealed an extensive role for *histone deacetylation* in Myc-mediated gene repression. We find that repression of Myc target genes such as *Id2* and *Gadd153* is associated with a loss of histone H4 acetylation. We also find that repression of both genes can be reversed by either deletion of MbIII or treatment of cells with histone deacetylase (HDAC) inhibitors, suggesting that active deacetylation of H4, mediated via MbIII, is important for Myc-driven repression. To determine which HDACs are responsible for this repression, we screened a panel of short-hairpin RNA (shRNAs) directed against each of the relevant HDACs. This analysis revealed that HDACs 3 and 4 are involved in repression of the *Id2* and *Gadd153* genes, an interesting result because both HDACs function in a common complex. We have confirmed the relevance of the functional data by showing that Myc interacts with HDAC3 and recruits it to chromatin, all of which occurs in a MbIII-dependent manner. These results reveal that one mechanism through which Myc represses transcription is via HDAC recruitment; an exciting finding because this is a previously unanticipated mode of Myc-mediated repression, and because HDAC inhibitors are showing promise in the clinic for the treatment of certain human cancers. In the future, we will probe how Myc interacts with HDACs, examine the contribution of this interaction to oncogenesis, and ask whether Myc is a relevant downstream target of therapeutic HDAC inhibition.

REGULATION OF TRANSCRIPTION BY THE UPS

Our original observation of the tight relationship between transcriptional activation and Ub-mediated destruction of transcriptional activators predicted that, at some level, components of the Ub–proteasome system would be involved in transcriptional regulation. During the past several years, work from a number of groups, including our own, has supported this prediction. For example, we have found that Ub ligases can be recruited to sites of transcription, where they perform essential steps in gene activation, including driving postinitiation changes in the entourage of RNA-polymerase-interacting proteins. Other groups have found that Ub ligases can promote coactivator exchange at promoters in vivo, as well as targeting stalled RNA polymerases for destruction to clear the path for successful gene transcription following DNA-damage-mediated transcriptional arrest. Moreover, ubiquitylation of histones has been linked to transcriptional repression and shown to participate in the regulation of covalent patterns of histone modifications. Our ongoing studies in this area continue to focus on the role of activator ubiquitylation, but we are also studying ubiquitylation of RNA polymerase and histones and the relationship between the Ub system and pre-mRNA processing.

We are particularly interested in understanding how histone ubiquitylation influences the transcriptional status of chromatin. In *S. cerevisiae*, the prototypical histone ubiquitylation event involves H2B, which has a single Ub group attached to a lysine residue 123 (K123) within the protein. Monoubiquitylation of H2B at this site is involved in a number of processes including activation of gene expression, gene silencing, and mitosis. A few years ago, we showed that ubiquitylation of H2B at K123 is also required for recruitment of proteasome subunits to chromatin, which in turn promotes SAGA (Spt-Ada-Gcn5-acetyltransferase) coactivator recruitment and histone H3 methylation. Interestingly, the proteasome binds only weakly to mono-Ub moieties, suggesting that recruitment of proteasome subunits to chromatin is indirectly modulated by histone monoubiquitylation or that perhaps the ubiquitylation status of H2B is more complex than previously thought.

To address the latter issue, we used a directed approach to probe the extent of H2B ubiquitylation in yeast cells. This analysis resulted in the surprising discovery that H2B is extensively polyubiquitylated (Fig. 1). Polyubiquitylation of H2B occurs within the context of chromatin and is not associated with H2B destruction. We have found that there are at least two distinct modes of H2B polyubiquitylation: One that occurs at K123 and depends on the Rad6/Bre1 ubiquitylation machinery, and another that occurs on multiple lysine residues and is catalyzed by an uncharacterized ubiquitin ligase(s). Interestingly, we also find these ubiquitylation events are under the influence of different combinations of Ub-specific proteases, suggesting that they have distinct biological functions. These results raise the possibility that the biological effects of ubiquitylation of H2B are exerted via ubiquitin chains, rather than a single Ub group. Our future research in this area will aim at identifying the cellular machinery responsible for these noncanonical ubiquitylation events, delineate the effects of the different Ub chains and linkages, and determine how the poly-Ub chains we describe on H2B contribute to proteasome subunit interaction with chromatin.

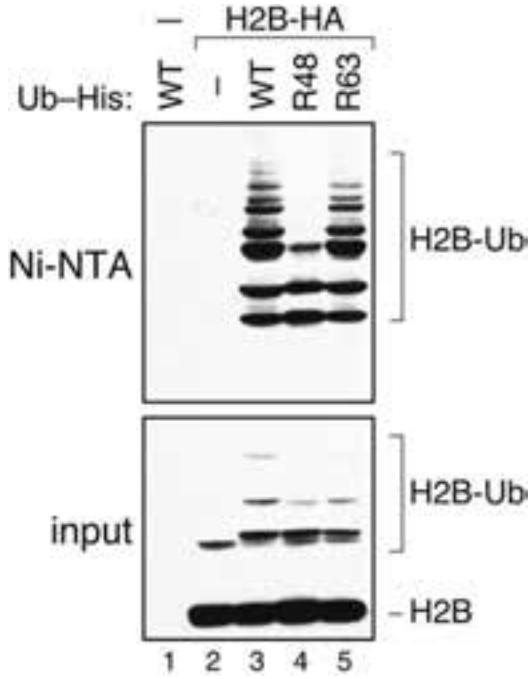

FIGURE 1 Histone H2B is polyubiquitylated. Yeast cells were engineered to express either His-tagged wild-type or mutant (K48R or K63R) nonremovable (G76A) ubiquitin, together with either Myc-tagged (*lane 1*) or HA-epitope-tagged H2B (*lanes 2–5*). Cells were lysed, and HA-tagged proteins present in the total lysate ("input") or His-purified ("Ni-NTA") material were detected by western blot. The position of free H2B is indicated, as is the position of the H2B-Ub conjugates. Note that the monoubiquitylated form of H2B-HA appears as a doublet in the input; we presume that this corresponds to H2B-HA that is monoubiquitylated with either endogenous Ub (*lower band*) or the slightly larger His-Ub.

PUBLICATIONS

Pal B., Chan N.C., Helfenbaum L., Kaeling T., Tansey W.P., and Gething M.-J. 2007. SCF^{Cdc4}-mediated degradation of the Hac1p transcription factor regulates the unfolded protein response in *Saccharomyces cerevisiae. Mol. Biol. Cell.* **18:** 426–440.

CANCER: GENETICS

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

Michael Wigler's group uses comparative genome hybridization (CGH) analysis to study cancer and human genetic disorders. Their method, a microarray-based technique called ROMA (representational oligonucleotide microarray analysis) that arose from an earlier technique called RDA (representational difference analysis), reveals copy-number variations (CNVs), the deletions and duplications which underlie cancer, many genetic disorders, and evolution. A variant called MOMA (methylation-specific oligonucleotide microarray analysis), developed with Rob Lucito, is used to explore the role of DNA methylation in cancer and genetics. The group focuses on breast cancer and leukemias with the aim of pinpointing genes that cause cancer and discovering mutation patterns that predict treatment response and outcome. With Jonathan Sebat, they have focused on autism, showing that spontaneous mutation has a far greater role in that disorder than previously suspected. Together with Kenny Ye, they have developed a new theory of autism's genetic basis that explains otherwise bewildering patterns of inheritance. Their theory may be relevant to other disabling genetic disorders.

Gregory Hannon has had a central role in the study of RNA interference (RNAi), a process by which double-stranded RNA (dsRNA) molecules induce gene silencing. Hannon is credited with the discovery of two enzymes, called Dicer and Slicer, critical in the RNAi machinery. (The work on Slicer was performed in collaboration with Leemor Joshua-Tor.) Hannon's lab has led the way in using RNAi to study cancer biology and genetics. They have generated a library of short-hairpin RNAs that they and other researchers are now applying broadly in gene-silencing studies. The short-hairpin molecules are particularly noteworthy, enabling researchers to get around a major problem in putting RNAi to work experimentally. Hannon's approach enables researchers to use RNAi to switch genes on and off in vivo and to make inferences about gene function by observing model systems. His lab is using this method, among other techniques, to identify new targets for cancer therapy.

Scott Lowe's lab studies cancer gene networks and determines how genetic lesions affecting these networks contribute to tumor development and resistance to cancer therapy. This year, Lowe and colleagues continued to study the process of cellular senescence, a potentially powerful mechanism for suppressing tumors, and showed that senescent cells activate a form of immune surveillance that might be harnessed therapeutically. In related work, Lowe has adapted RNA interference technology to produce animal models in which genes can be switched on and off in a spatial, temporal, and reversible manner and used this to study how correcting genetic defects that contribute to cancer can lead to tumor regression. Finally, Lowe continues to use an integrative approach to cancer gene discovery that combines genomic information and mouse modeling to rapidly identify and characterize new cancer genes and potential therapeutic targets.

In recent years, Robert Lucito, in collaboration with Michael Wigler, has had an important role in developing technologies, including RDA, ROMA, and CGH (comparative genome hybridization), that have proven to be valuable to cancer researchers at Cold Spring Harbor and beyond. Lucito has conducted studies using these techniques to detect copy-number changes in large sets of human ovarian and pancreatic cancer tissue samples. This year, the lab turned its attention to epigenetics, and specifically to the study of methylation throughout the genome. When methyl groups in sufficient numbers attach to cytosine bases in DNA, the packing of DNA into the chromosomes can be altered, reducing the levels at which the methylated genes are expressed. Under circumstances that Lucito is exploring, this may be critical in certain cells, a means by which normally protective tumor-suppressor genes are turned off, rendering the cells tumorigenic.

Scott Powers's work focuses on gene mutations that cause cancer and factors that influence responses to specific anticancer drugs. 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. Having successfully applied this approach in liver and colon cancers, the lab recently turned its attention to lung cancer. This year, Powers and David Mu discovered three contiguous genes on human chromosome 14 that interact to reactivate an early fetal gene expression pattern that results in cancer. They identified the mutation that triggers this abnormal reactivation—which occurs in 20% of non-small-cell lung cancers—and showed that if these genes are turned off, the cancer retreats.

Alea Mills is studying genetic pathways important in cancer and aging, identifying the genetic players and determining how aberrations in their functions lead to human disease. This year, Mills's "chromosome engineering" efforts culminated in the identification of an important tumor suppressor gene that had eluded investigators for three decades. Genomic analyses had clearly identified a human chromosome called *1p36* that was frequently deleted in neural, epithelial, and hematopoietic malignancies, indicating that the region harbored a tumor suppressor. The tumor-suppressing gene, ultimately identified as *Chd5*, was shown by Mills to regulate an extensive network. The epigenetic role of *Chd5* in development, cancer, and stem cell maintenance is currently being investigated. The Mills lab is also studying a protein called p63, which causes developmental defects, protects from tumorigenesis, triggers cellular senescence, and leads to accelerated aging in vivo.

Much of what is known about genome plasticity and genetic instability has been gleaned from comparative studies of sequence variations in model organisms, measured over evolutionary timescales. Less is known about genetic variation over more rapid time intervals, within normal populations and organisms. This has been the subject of CSHL Fellow Ira Hall's research. Using a mouse model system, he has investigated properties of DNA-copy-number fluctuation using comparative genome hybridization. This work has indicated the questions that will drive Hall's research in the coming years: How do new copy-number variations arise? What are the prevalent mechanisms of duplication and deletion? Are different chromosomal regions or classes of DNA sequences more variable than others?

The identification of cancer stem cells with vital roles in tumor progression, maintenance, and recurrence has suggested that at least some cancers arise from malfunctioning developmental programs. CSHL Fellow Patrick Paddison's research, which focuses on how molecular pathways promote or contain "stemness," may therefore provide insight into cancer biology and point to new therapeutic strategies. Using in vivo functional-genetic approaches, Paddison is identifying genes required for self-renewal and lineage specification in embryonic and adult stem cells in humans and rodents. He seeks to determine whether these genes are involved in stem-cell-driven cancers.

RNA INTERFERENCE MECHANISMS AND APPLICATIONS

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H.S. Bender
S. Boettcher
J. Brennecke
A. Canela
K. Chang
S. Cheloufi
K. Claudio-Campos
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A. Molaro
M. Mosquera
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M. Rooks
N. Rozhkov
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D. Siolas
C. Smith
V. Sotirova
O. Tam
V. Vagin
Y. Yang
R. Zhou

We continue to work across our two core areas of cancer genetics and small RNA biology, with each benefiting from the insights of the other. The signature of our approach remains comparative biology, working across multiple organismal systems to extract the most fundamental aspects of the problems that we study. We apply the tools of genetics, genomics, biochemistry, and increasingly computational biology to try to identify the inherent vulnerabilities of cancer cells and to understand the biology of small RNAs.

This year, the lab continued its tradition of recruiting and training young scientists. We were joined by four outstanding postdocs—Andres Canela, Astrid Haase, Vihra Sotirova, and Vasily Vagin—who will strengthen our cancer, genomics, and small RNA programs. Yaniv Erlich and Colin Malone entered the laboratory from the Watson School of Biological Sciences and have already made tremendous impacts. Michelle Rooks joined our genomics efforts, Maria Mosquera joined to support our mouse group, and Hana Mizuno returned to the laboratory's cancer program. We had the pleasure of hosting a number of visiting scientists who bring their own perspective and experience to our work. Rui Zhou, Ben Czech, and Nick Rozhkov joined the *Drosophila* group, and Hannah Bender, Carly Smith, Clare Rebbeck, and Karla Claudio-Campos joined the Tasmanian Devil project. Antoine Molaro is helping to push our understanding of piRNA systems in mice. We benefited from the help of undergraduate students, Edie Davis, YoungJoo Yang, and Anna Jankowska. This year has also seen departures: Lin He left to accept a faculty position at the University of California, Berkeley; Catherine Schlingheyde, a phenomenal high school student, entered Case Western Reserve University; and Tom Gingeras, a visiting scientist, left us to become a Professor at CSHL.

Summaries of our individual projects and programs appear below.

Developmental Regulation of piRNAs in the Mouse Male Germ Line

A. Aravin

We have investigated the functions of Piwi proteins and associated small RNAs (piRNAs) in the mouse germ line. Originally, piRNAs were shown to be developmentally regulated and expressed starting at the pachytene stage of meiosis in the male germ line. To gain a comprehensive picture of the role of the Piwi/piRNA pathway in germ cells, we investigated the expression of the three mouse Piwi members (Miwi, Mili, and Miwi2) throughout spermatogenesis. We produced transgenic animals that express each Piwi family member tagged with green fluorescent protein (GFP) and myc/FLAG tags. Both Mili and Miwi2 are expressed in male gonads during embryogenesis. The onset of Mili expression (12.5 dpc) precedes that of Miwi2 (14.5 dpc). Starting at E14.5, both proteins are expressed in germ cells simultaneously; Mili is localized exclusively in cytoplasm and Miwi2 is predominantly nuclear with few prominent cytoplasmic granules. Miwi2 expression ceases soon after birth, whereas Mili continues to be expressed until the haploid round spermatid stage.

To gain insight into piRNA populations bound to each protein, we immunoprecipitated protein–piRNA complexes and cloned and deeply sequenced isolated RNAs. Both proteins predominantly bound to small RNAs derived from long terminal repeat (LTR)- and non-LTR-containing retrotransposons. Although Mili has a preference for sense-strand piRNAs, Miwi2 prefers antisense sequences. Furthermore, piRNAs bound to these two proteins show features which suggest that they were processed as a result of amplification loop with each respective Piwi member being responsible for the for-

mation of the 5′ ends of new piRNAs that preferentially bind to other proteins. Molecular interactions between the two proteins is also confirmed by delocalization of Miwi2 from nucleus in Mili knockout animals.

The narrow window of Miwi2 expression during embryogenesis corresponds to the time when methylation imprints of transposable elements are established in male germ cells. Furthermore, methylation of L1 elements is greatly reduced in Miwi2 KO animals. To further understand the interaction between Piwi/piRNA and DNA methylation pathways, we investigated piRNA profiles in Dnmt3L KO animals that are unable to establish methylation of retrotransposon sequences. We found that retrotransposon-derived piRNAs are in fact more abundant in Dnmt3L KO animals as compared to wild type. This finding indicates that derepression of retrotransposons in Dnmt3L mutants leads to increased production of piRNAs and places the Piwi/piRNA pathway as an upstream determinant of DNA methylation.

Small RNA-directed DNA Methylation in Mammals

A. Girard

The Piwi family of proteins and their Piwi-interacting RNA (piRNA) partners have a central role in targeting transposable elements for silencing in many animals. In a first phase, transposon-derived piRNAs are specifically generated, amplified, and then bound to a Piwi effector protein. Through piRNA–target base-pair interactions, transposon transcripts are recognized by the Piwi-piRNA complex, which can then serve a scaffold for recruitment of chromatin-modifying enzymes. In mammals, transposons are silenced by both DNA methylation and repressive histone modifications. DNA methylation on several of those repetitive sequences is lost in Piwi mutant mice. I am currently trying to understand the requirement for Piwi proteins in DNA methylation (and potentially histone modifications) on a genome-wide scale, as well as the mechanism by which Piwi proteins trigger chromatin changes on repetitive elements.

Roles for piRNAs in Transcriptional Gene Silencing in *Drosophila*

K.F. Toth, A. Aravin

Mobile genetic elements populate the genomes of nearly every living organism, and their potential negative effects necessitate the development of strategies for transposon control. This is especially critical in the germ line, where mutations caused by transposon activity would accumulate with each passing generation. Silencing of genomic repeats, including transposable elements, in *Drosophila melanogaster* is mediated by a subclass of the Argonaute proteins, termed Piwi proteins and associated piRNAs. Piwi proteins are expressed exclusively in the germ line, and genetic studies have implicated Piwi proteins in germ-line integrity. Their associated piRNAs are 26–30 nucleotides long and are produced from discrete loci in the genome. They act as guides for the identification of targets of the Piwi proteins.

Some of the piRNA-based silencing is accomplished by the target cleavage activity of Piwi proteins; however, some data indicate a function of Piwi on the transcriptional level. To address this possibility, we have asked the following questions:

- Does the piRNA pathway have any role in transposon regulation on the chromatin/transcriptional level?
- Is Piwi directly involved in transcriptional repression of transposons by associating and altering chromatin?

To answer the first question, we have carried out chromatin immunoprecipitation (ChIP) experiments with different histone marks and chromatin-associated factors from wild-type flies and strains deficient for some of the piRNA pathway proteins. The obtained DNA was analyzed by quantitative polymerase chain reaction (Q-PCR) and by cloning and deep sequencing. Preliminary results indicated that at least some of the transposons show altered chromatin marks in the mutants, indicating some role of the pathway on transcriptional regulation.

To test whether Piwi is directly interacting with chromatin, we have performed ChIP experiments using strains with tagged Piwi. In addition, fluorescent microscopy was used to determine the subnuclear localization of Piwi. Both the microscopic and biochemical data indicate that Piwi is associated with chromatin and that this association is dependent on the underlying genomic sequence.

Endogeneous Small RNA Pathways in *Drosophila*

J. Brennecke, C. Malone, B. Czech

Small RNA-directed silencing processes or RNA interference pathways participate vitally in chromatin regu-

lation, repression of gene expression, viral defense, and silencing of selfish genetic elements. At the core of each pathway lies an Argonaute protein, which in its active form is loaded with a single-stranded 21–30-nucleotide small RNA, guiding the Argonaute protein complex to RNA targets with complementary nucleotide signatures. A variety of small RNA generating pathways act upstream of distinct Argonautes, resulting in the specialization of Argonaute effector complexes for the identity of their targets. We are interested in the in vivo functions of Argonaute proteins in *Drosophila*, especially those acting to protect the genome against selfish genetic elements such as transposons.

Besides our recent insights into the Piwi pathway, which seems to have specifically evolved to fight transposable elements, our recent work indicates that a classical RNA interference pathway involving small interfering RNAs (siRNAs) loaded into Ago2 have an important role in silencing transposons, likely in the germ line and soma. The systematic analysis of Ago2-bound small RNAs led to the discovery of multiple classes of endogenous siRNAs, which seem to participate in viral defense, transposon silencing, and gene regulation. Our combined interest is in the systematic deciphering of piRNA and siRNA-directed silencing pathways in the *Drosophila* germ line, from biogenesis to mode of action and in vivo function.

The Cell Biology of piRNAs in *Drosophila*

M. Dus

Animal and plant genomes contain large amounts of transposons and other types of selfish DNA, comprising up to 50% or more of their total content. These elements are often considered "junk DNA," but the accumulation of repetitive elements at centromeres and telomeres suggests beneficial or evolutionarily important roles of transposons for chromosome biology. Nevertheless, the deleterious consequences of their activity, especially in germ-line cells, represent an ongoing challenge for the host to keep uncontrolled replication of these parasitic elements under control. With the expansion of selfish genetic elements, genomes have evolved elaborate defense mechanisms to suppress such proliferation.

In *Drosophila*, the female germ line is enveloped by a monolayer of somatic cells called follicle cells. In both germ-line and follicle cells, protection of the germ line from parasitic genetic elements is mediated by Piwi proteins and small 28-mer RNAs (piRNAs) derived from transposons. A transposon-responsive amplification loop involving the three *Drosophila* Piwi clade proteins, Aubergine, Piwi, and Ago3, is required to mount a robust defense. However, proteins other than Aubergine, Piwi, and Ago3 are required to control transposon mobilization, such as the RNA helicases, Armitage, and Spindle-E, and the novel proteins Maelstrom, Zucchini, Squash, and Krimper. The aim of our work is to dissect the role of these proteins in transposon control, specifically to assign them distinct positions in the pathway. With high-throughput piRNA sequencing, localization studies, mass spectrometry, and Q-PCRs, we have observed perturbations in both small RNA profiles, transposon activation and subcellular localization, for all proteins under investigation, indicating that each of them affects the pathway at a different and discrete step. Our results indicate that the defense mechanisms that control transposons in the germ line are markedly different from those in the soma. This suggests that in both compartments, evolutionary forces have selected for defense mechanisms optimized to control transposons active in specific cell types.

Probing the Biochemistry of piRNA Pathways in Flies

A. Haase

I am working to establish an in vitro system that allows us to assay for specific events of piRNA biogenesis and to then purify and characterize components involved in these processing steps. Our in vitro system will consist of recombinant Piwi proteins (produced in collaboration with Leemor Joshua-Tor's lab here at CSHL) or immunoprecipitates, synthetic and in-vitro-transcribed piRNA precursors, and different cell extracts as sources for the processing activities. Using different experimental strategies, we aim to purify and identify proteins involved in piRNA biogenesis, especially components of the 3′-end processing machinery, giving rise to piRNAs of distinct size. In addition, we will investigate the impact of different 5′ piRNA terminal nucleotides on their sorting into distinct Piwi complexes. Finally, we want to analyze the initiation phase of piRNA biogenesis with the aim of identifying the initial determinants of piRNA processing.

Pseudogene-derived Small Interfering RNAs in Mouse Oocytes

O. Tam, A. Aravin

Three small RNA classes in mammals regulate expression of genes or repeat elements: small interfering

RNAs (siRNAs), microRNA (miRNAs), and Piwi-interacting RNAs (piRNAs). In most mammalian tissues, miRNAs are responsible for the bulk of small RNA-mediated gene regulation through translational inhibition. In addition to miRNAs, the male germ line also possesses another highly abundant class of small RNAs (piRNAs), which appears to be responsible for the silencing of repeat elements (such as retrotransposons) in the mouse genome through an as yet unknown mechanism.

These observations, however, raised two conundrums: (1) Although the male germ line requires piRNA-mediated silencing (as shown by male sterility in mutants of the mammalian Piwi proteins), the females are fertile and unaffected. Are piRNAs not found or required in the female germ line? (2) siRNA-mediated silencing via target cleavage remains active in mammals (as demonstrated by gene knockdown by exogenous siRNA), yet no endogenous siRNAs have been reported. What then is the evolutionary requirement for maintaining cleavage activity in mammals (which is conserved from platypus to humans)?

A recent deep-sequencing study into the small RNA population of mammalian female germ line (oocytes) has provided some answers to these conundrums. Using Solexa/Illumina sequencing technology, we were able to sequence and characterize the small RNA populations in oocytes. We managed to obtain a profile of oocyte-expressed miRNAs, as well as identifying a piRNA population that targets repeat elements (not previously reported in the female germ line). The most interesting observation, however, is the identification of a class of small RNAs that resemble endogenous siRNAs. A group of these "siRNAs" targets repeat elements in the genome, potentially having a role in silencing them in tandem with piRNAs (thus providing a possible explanation for the fertility of female mice lacking mammalian Piwi proteins).

A second more interesting group appears to be generated through the interaction of a genic transcript and an antisense transcript generated from a corresponding pseudogene. We hypothesize that these two transcripts form a double-stranded RNA, which upon Dicer processing, leads to siRNAs targeting the corresponding gene. Interestingly, these "siRNA"-generating genes are up-regulated in the absence of Dicer, raising the possibility that their regulation could be dependent on siRNA production. The discovery of these endogenous siRNAs will change the current model of small RNA-mediated silencing in mammals and also provide the first evidence of the evolutionary requirement of cleavage-mediated RNA interference.

Specialization of Argonaute Proteins in Mice

S. Cheloufi, O. Tam, T. Karginov

miRNAs and exogenously provided siRNAs interact with four mammalian Ago proteins, Ago1 through Ago4. Ago2 is the only Argonaute protein that has been demonstrated to have a slicer activity responsible for cleaving target mRNAs. We have shown that Argonaute2 is essential during mouse embryogenesis. Argonaute2-deficient mice are lethal around mid-gestation with heart and neural tube malformations. However, little is known about the differences between Argonautes during development. Individual knockouts of Ago1, Ago3, and Ago4 and double- and triple-knockout combinations are viable and fertile and do not reveal any obvious developmental abnormalities.

To investigate the role of Ago2 during embryonic development, we have generated Ago2-null embryonic stem (ES) cells that fail to mount an siRNA-mediated gene-silencing response. Ago2-null ES cells contribute to the whole embryo at E10.5. Further time points of chimeric studies will allow us to dissect the developmental potential of the Ago2-deficient ES cells. To identify small RNA partners of the Argonautes during embryonic development, we have constructed small RNA libraries from mid-gestation embryos and extraembryonic tissues and generated an miRNA expression profile of embryonic and extraembryonic tissues. We also generated miRNA profiles in Ago2-null ES cells. Comparison with a wild-type miRNA profile allowed us to identify several miRNA clusters that were specifically reduced in the absence of Ago2. One third of all miRNAs in extraembryonic tissues is derived from one of these clusters, although it shows low abundance in the embryo. To further characterize the function of the slicing activity of Ago2 during development. We have generated an Ago2 catalytically mutant mouse by mutating an essential residue within the catalytic domain of ago2. Profiling of the small RNA expression during embryogenesis together with analyzing the Ago2 mutants will help us understand how Ago2 functions during mouse embryogenesis.

microRNAs and Their Role in Tumorigenesis

X. He, L. He

To date, most cancer research has focused on the alternations of protein-coding genes. However, recent evidence has suggested that alternations of noncoding

RNA, particularly microRNAs (miRNAs), can also contribute to tumor formation. For example, it has been reported that a polycistronic miRNA cluster, *mir-17-92*, cooperates with c-*myc* to accelerate tumor growth by repressing apoptosis in a mouse model of B-cell lymphoma. These data provided some of the first evidence that changes in noncoding RNAs could contribute to human cancer. On the other hand, there is also evidence that miRNAs can function as tumor suppressors since some miRNAs, such as *mir-15*, *mi-16*, and *let-7*, exhibit significant reductions in cancer.

We have identified *mir-34* family miRNAs, *mir-34a*, *b*, and *c*, as direct transcriptional targets of *p53*, a prevalent tumor suppressor gene. We have demonstrated that ectopic expression of *mir-34* in both primary and tumor cell lines can induce growth arrest through repression of cell cycle genes, including *cdk4*, *cyclinE2*, and *met*, and we have shown in liver tumor models that tumor cells overexpressing *mir-34a* have a disadvantage in tumor initiation and maintenance. This result, consistence with the fact that deletion of *mir-34a* has been found in many cancer types, indicates that *mir-34a* is a potential tumor suppressor. In the coming year, we will further examine the biological functions of miR-34 miRNAs in the p53 tumor suppressor network, focusing on identifying the miR-34's targets, validating the potential role of miR-34 as a tumor suppressor, and generating miR-34 knockout mice to examine the consequence of its loss of function.

Assessing the Therapeutic Potential of Small RNAs

F. Rollins

Recent advances in RNAi technologies have produced a tremendous impact on cancer biology. New tumor suppressors and oncogenes have been identified, and using RNAi, we have tools to further understand the gene networks that underlie and drive processes in cells. One drawback is the inability to use RNAi in vivo. I have been working to further develop tools and methods for using RNAi in vivo, both as a means for validation of newly identified genes and to test potential therapeutics. I have been developing a reporter for RNAi, and once this project is completed, we will be able to test different RNAi vehicles for efficacy and tissue distribution. In this way, we can screen compounds and develop models. The information generated by the reporters will be used to understand which compounds function best in certain tissues and to tailor experiments to specific areas and genes of interest within animals.

Toward the $1000 Genome

Y. Erlich

The advent of single-molecule sequencing has transformed the study of genomes, both in describing their transcriptional output and in the cataloging individual genetic variation. Presently, the power of these technologies is limited partly by short-sequence read lengths and error rates higher than those seen with conventional sequencing. To maximize both sequence output and accuracy, we have probed the underlying sources of error in the Illumina 1G platform. We find that the major limitation of this method is the accumulation of noise that gradually decreases the accuracy of base calling. Noise can be separated into phasing, loss of signal intensities, and a convergence of signals from base-specific fluorophores. We have developed a novel base-calling algorithm that combines optimized phase modeling and machine learning. Implementation of this program increases the number of correct sequence reads by 30% and significantly extends the useful read length achieved on the platform.

Small RNA Pathways in Marsupials and Monotremes

E.P. Murchison, H.S. Bender, C. Rebbeck, C. Smith, K. Claudio-Campos

Our group is interested in understanding the molecular basis of Tasmanian devil facial tumor disease (DFTD), a transmissible cancer affecting Tasmanian devils. First observed in 1996, DFTD has had a devastating toll on the Tasmanian devil, a marsupial carnivore native to Tasmania. Karyotypic similarities between DFTD tumors suggested that the disease is transmitted as an allograft. Studies of microsatellite and mitochondrial variability revealed that all DFTD tumors are indeed genetically identical, indicative of a common origin. To ascertain the tissue of origin and molecular pathogenesis of DFTD, we have cloned and sequenced miRNA libraries from DFTD and are sequencing full-length devil cDNA libraries.

In addition, as part of the platypus genome consortium, we have cloned, sequenced, and characterized small RNAs in the platypus. The platypus and echidna are the only extant egg-laying mammals, known as monotremes. These animals have unique morphology and development, displaying both mammalian and reptilian features. Interestingly, this evolutionary intersec-

tion is also apparent in the genome sequence, and we found that the microRNAome of monotremes bears resemblances to those of both mammals and chicken. The platypus also has unusual sex chromosomes, with five X and five Y chromosomes segregating through a meiotic chain in the male germ line. We discovered a large cluster of monotreme-specific miRNAs on one of the X chromosomes that is extremely fast-evolving and is expressed in testis. This cluster may have a role in monotreme reproductive biology.

Genetic Strategies to Identify New Targets for Cancer Therapy

K. Chang, K. Marran, J. Silva

By virtue of their accumulated genetic alterations, tumor cells may acquire vulnerabilities that create opportunities for therapeutic intervention. We have devised a massively parallel strategy for screening shRNA collections for stable loss-of-function phenotypes. We have assayed from 6,000 to 20,000 shRNAs simultaneously to identify genes important for the proliferation and survival of five cultured mammary cell lines. Lethal shRNAs common to these cell lines targeted many known cell cycle regulatory networks. Cell-line-specific sensitivities to suppression of protein complexes and biological pathways also emerged, and these could be validated by RNAi and pharmacologically. These studies establish a practical platform for genome-scale screening of complex phenotypes in mammalian cells and demonstrate that RNAi can be used to expose genotype-specific sensitivities.

A Cytoskeletal Regeulator as a Tumor Suppressor

J. Silva, K. Marran

Complete sequencing of the human genome has provided information on potentially all expressed genes and has opened the door to the next scientific challenge: to assign a specific function to every gene. RNAi has recently emerged as a powerful genetic tool to conquer this challenge by performing loss-of-function studies. During the last several years, we have been developing a highly efficient miRNA-based shRNA library that facilitates genome-wide loss-of-function studies to delineate molecular pathways.

Additionally, we have coupled this technology with high-resolution genomic analysis (with the Wigler lab here at CSHL) to identify novel tumor suppressor genes recurrently deleted in human epithelial cancers. This approach has led to the finding that suppression of Cyfip1/Sra1, a component of the WAVE complex that regulates cytoskeleton dynamics, disturbs normal epithelial morphogenesis in vitro and cooperates with oncogenic Ras to produce invasive carcinomas in vivo, resulting from impaired cell adhesion and increased motility. In addition, reduced expression of Cyfip1/Sra1 was found to be associated with poor prognosis in breast and colon cancer patients. These results, have led me to propose that Cyfip1/Sra1 is a putative tumor suppressor in epithelial cancers.

Focal Genome Resequencing to Identify Breast Cancer Susceptibility Determinants

E. Hodges, J. Silva, M. Rooks [in collaboration with D. McCombie, Cold Spring Harbor Laboratory]

Recently, we and other investigators have reported enrichment techniques for genomic target capture and directed resequencing of large subsets of the nonrepetitive genome. Our strategies combine hybrid selection, either on microarrays or in solution, with massively parallel sequencing on the Illumina Genome Analyzer. These methods are already proving to be highly competitive in both efficiency and cost in contrast to other target selection techniques including long-range and multiplex PCR. Currently, we are developing optimized platforms for in situ capture of protein-coding sequences to enable extensive high-resolution surveys of the mutational state of specified regions in multiple affected individuals. Our goal is to reduce the amount of starting material required while improving both target specificity and base-pair coverage. This technology is robust, reproducible, and portable to other laboratories.

We are currently performing focused resequencing on candidate regions linked to familial breast cancer. Mutations involving two high-penetrance breast cancer susceptibility genes, *BRCA1* and *BRCA2*, account for nearly 30% of familial breast cancer. Although lower-risk genes have been identified, mainly through a priori candidate gene approaches, efforts to account for the remaining 70% of familial breast cancer cases (referred to as BRCAX families) have been fruitless, and the discovery of additional predisposition genes continues to be difficult. For this project, genome-wide linkage scans performed across multiple BRCAX families identified

three candidate regions representing about 140 genes. To further characterize these regions, we have designed capture arrays tiling all coding exons within these regions and are using them to search for mutations that represent high-penetrance risk factors.

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DISSECTING TUMOR SUPPRESSOR GENE NETWORKS CONTROLLING APOPTOSIS AND SENESCENCE

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Apoptosis is a genetically controlled form of cell death that is important for normal development and tissue homeostasis. Senescence is a permanent form of cell cycle arrest that can be triggered in response to cellular stress. Both processes can efficiently eliminate incipient cancer cells and are often disabled in malignant tumors. Moreover, radiation and many chemotherapeutic agents can induce either apoptosis or senescence in tumor cells, and so the ability of tumor cells to mount an apoptotic or senescence response may influence the outcome of cancer therapy in patients. The goal of our research is to understand how cancer genes control apoptosis and senescence in normal cells, and how mutations that disrupt these processes impact tumor development and cancer therapy. Ultimately, we hope to exploit this information to identify more effective and less toxic strategies for treating cancer.

Control of Apoptosis by Oncogenes and Tumor Suppressor Genes

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Normal cells possess intrinsic tumor suppressor mechanisms that limit the consequences of aberrant proliferation. For example, deregulated expression of the oncogene c-*myc* or disruption of the *Rb* pathway in normal cells can force aberrant S-phase entry and predispose cells to apoptotic cell death. This increased sensitivity to apoptosis acts through both *p53*-dependent and -independent pathways and potently limits tumor development. We have previously shown that oncogenes can engage the *ARF-p53* pathway to promote apoptosis and that disruption of this pathway cooperates with oncogenes to transform normal cells in vitro and promote tumorigenesis in vivo. We are currently interested in identifying additional components of these programs and understanding how they function in a "tumor suppressor network."

Cbx7 is a chromobox family protein and a component of the polycomb repressive complex 1 (PRC1) that extends the life span of cultured epithelial cells and can act independently of Bmi-1 (another PRC1 component) to repress the *Ink4a/Arf* tumor suppressor locus. To determine whether *CBX7* might be oncogenic, we examined its expression pattern in a range of normal human tissues and tumor samples. CBX7 was expressed at high levels in germinal-center-derived follicular lymphomas, where elevated expression correlated with high c-*myc* expression and a more advanced tumor grade. By targeting *Cbx7* expression to the lymphoid compartment in mice, we showed that *Cbx7* can initiate T-cell lymphomagenesis and can cooperate with c-*myc* to produce highly aggressive B-cell lymphomas, in part, by suppressing apoptosis. These data identify CBX7 as the first chromobox protein causally linked to cancer development and may explain, at least in part, the low frequency of *INK4a/ARF* mutations observed in human follicular lymphoma (Scott et al. 2007).

Another area of interest in our group relates to the impact of survival signaling on oncogenesis. We previously used the Eμ-*myc* lymphoma model to demonstrate that the translation initiation factor *eIF4E* is a potent oncogene in vivo and can promote resistance to certain cancer therapies. Using the same model, we recently showed that the oncogenic activity of eIF4E correlates with its ability to become phosphorylated on Ser-209 and activate translation (Wendel et al. 2007). Furthermore, constitutively activated MNK1, the eIF4E Ser-209 kinase, promoted tumorigenesis in a manner similar to that of eIF4E, whereas a dominant-negative *mnk* mutant inhibits the in vivo proliferation of tumor cells driven by mutations that deregulate translation. Phosphorylated eIF4E promotes tumorigenesis primarily by suppressing apoptosis, and, accordingly, the antiapoptotic protein Mcl-1 was identified as one target of both

phospho-eIF4E and MNK1 that contributed to tumor formation. These results provide insight into how eIF4E contributes to tumorigenesis and pinpoints an avenue for protein translational control that may be suitable for therapeutic intervention.

Control of Cellular Senescence

A. Chicas, Y. Chien, M.V. Krizhanovsky, M. McCurrach, W. Xue, L. Zender [in collaboration with L. He, X. He, and G. Hannon, Cold Spring Harbor Laboratory; E. Hernando, New York University; and C. Cordon-Cardo, Columbia University]

Cellular senescence was originally described as the process that accompanies replicative exhaustion in cultured human fibroblasts, and it is characterized by a series of poorly understood markers. Senescent cells remain metabolically active, but they are unable to proliferate and display changes in gene expression that could alter tissue physiology. As such, they are genetically "dead" and cannot contribute to tumor development. Although "replicative" senescence is triggered by telomere attrition and can be prevented by reactivating telomerase, a similar endpoint can be produced acutely in response to activated oncogenes, DNA damage, oxidative stress, and suboptimal cell culture conditions. These observations led us to propose that senescence acts in parallel to apoptosis as a cellular response to stress that suppresses tumorigenesis and mediate responses to chemotherapy. On the basis of this analogy, much of our work on senescence is guided by our past experience on apoptosis.

Our laboratory was the first to demonstrate that deregulated mitogenic oncogenes could drive cells into a senescent state, thereby preventing oncogenic transformation, and that senescence could contribute to the outcome of chemotherapy in vivo. On the basis of the hypothesis that senescence is an important tumor suppressive mechanism in vivo, our laboratory continues to study the mechanisms and biology of cellular senescence.

This year, we conducted a series of experiments to show that *p53*-deficient liver carcinoma cells undergo senescence following *p53* reactivation in vivo (Xue et al. 2007). Remarkably, although senescence is a cytostatic program, the tumors underwent massive regression as a result of an attack by the immune system on the senescent cells. Thus, despite the cytostatic nature of the senescence program, senescent cells can turn over in vivo. In addition, although it is established that chronic inflammation triggered by senescent stromal cells or other factors can promote tumorigenesis, our work illustrates how innate immune cells—when targeted against senescent tumor cells—can have antitumor effects as well. We suggest that therapeutic strategies that specifically harness these processes may represent a promising therapeutic approach. We are also exploring the interplay between senescent cells and the tissue microenvironment in other pathologies, including liver cirrhosis.

In collaboration with the Hannon laboratory here at CSHL, we also identified a microRNA (miRNA) component of the *p53* tumor suppressor network that controls senescence (He et al. 2007). Specifically, by comparing miRNA expression profiles between wild-type and *p53*-deficient cells, we identified a family of miRNAs, *mir-34a-c*, whose expression reflected *p53* status. Precursors of all three miR-34 family miRNAs are direct transcriptional targets of *p53*, whose induction by DNA damage and oncogenic stress requires *p53* both in vitro and in vivo. Ectopic expression of miR-34 induced cell cycle arrest in both primary and tumor-derived cell lines, consistent with the observed ability of miR-34 to down-regulate a program of genes promoting cell cycle progression. The *p53* network suppresses tumor formation through coordinated activation of multiple transcriptional targets, and miR-34 may act in concert with other effectors to inhibit inappropriate cell proliferation.

Modulation of Gene Expression Using RNA Interference

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RNA interference (RNAi) is a powerful method for suppressing gene expression in mammalian cells. Stable knockdown of gene expression can be achieved by continuous expression of synthetic short hairpin RNAs (shRNAs), typically from RNA polymerase III promoters. However, primary microRNA transcripts (pri-miRNAs), which are endogenous triggers of RNAi, are normally synthesized by RNA polymerase II (pol II). With Gregory Hannon here at CSHL, we previously showed that pol II promoters expressing rationally designed pri-miRNA-based shRNAs (shRNA^{mir}) produce potent, stable, and regulatable gene knockdown in cul-

tured cells and in animals, even when present at a single copy in the genome. In practice, this shRNA^{mir} vector system is remarkably similar to cDNA overexpression systems and should be a powerful tool for studying tumor suppressor gene function in cells and animals.

We explored the potential of using reversible RNAi to regulate endogenous gene expression in mice. As an initial step, we generated transgenic mice containing an inducible tet-responsive miR30-based p53 shRNA and crossed them to a variety of tet-*trans*-activator transgenic strains. Upon providing or omitting doxycycline (the tetracycline analog) in the drinking water, we could reversibly reduce *p53* expression in a tissue-specific manner. We are now confident that this system has wide-reaching potential as we showed that conditional knockdown of *p53* accelerated tumorigenesis and that *p53* reactivation led to tumor clearance (Dickins et al. 2007). This result not only demonstrates an important role for *p53* loss in lymphoma maintenance, but also implies that similar systems can be used to validate drug targets or particular pathways for therapeutic intervention. In principle, similar systems can be used to spatially and temporally control the expression of any endogenous gene. We are currently pursuing second-generation strategies to improve vectors and gene-targeting approaches.

In addition to characterizing individual genes, we are exploiting this in vivo system in a very powerful way to identify new tumor suppressor genes. By expressing pools of shRNAs in one animal, we can assess the role of knockdown of hundreds of genes concurrently. We are performing in vivo screens in both the *E*μ-*myc* lymphoma and hepatocellular carcinoma mouse models. Both screens are nearly complete and have led to the validation of candidate tumor suppressor genes that are currently being characterized.

Molecular Genetics of Chemotherapeutic Drug Sensitivity and Resistance

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Our laboratory has a long-standing interest in understanding genetic determinants that influence the cellular response to conventional chemotherapy with the long-term goal of identifying mechanisms of drug resistance and developing new drug targets. These efforts involve a combination of experiments using RNAi to characterize drug sensitivity and resistance genes, genomic approaches designed to identify genes that are linked to poor treatment responses in patients, and animal modeling studies to test new drugs and drug combinations that might circumvent drug resistance.

Topoisomerase poisons are chemotherapeutic agents that are used extensively for treating human malignancies. These drugs can be highly effective, yet tumors are frequently refractory to treatment or become resistant upon tumor relapse. Using a rapid pool-based RNAi screening approach and a well-characterized mouse model of lymphoma, we explored the genetic basis for heterogeneous responses to topoisomerase poisons in vitro and in vivo. These experiments identified *Top2A* expression levels as a major determinant of the response to the topoisomerase 2 poison doxorubicin and showed that suppression of *Top2A* produces resistance to doxorubicin in vitro and in vivo (Burgess et al., in press). Analogously, using a targeted RNAi approach, we demonstrated that suppression of *Top1* produces resistance to the topoisomerase 1 poison camptothecin, again leading to drug resistance in vivo. Although the resistance-promoting effects of topoisomerase suppression were specific to their corresponding poisons, *Top1* knockdown unexpectedly enhanced sensitivity to doxorubicin, leading to improved survival of tumor-bearing mice. These results highlight the potential utility of combining RNAi with mouse models to identify determinants of therapeutic outcome in vivo. They further suggest strategies for improving the effectiveness of topoisomerase poisons in the clinic by stratifying patients or developing new sensitizing drug combinations.

In complementary efforts, we are continuing a multi-institutional program to develop and use mouse models of acute myelogenous leukemia to identify molecular determinants of both conventional and targeted therapies, with the goal of translating this information to human patients. We are excited about the potential of the program, as it provides all of the elements necessary to validate the use of new mouse models as preclinical test systems and translate this information into clinical trials. We are also integrating experiments using mouse models, genomics, and RNAi methods in an attempt to increase the rate of discovery of genes that influence the sensitivity and resistance of tumor cells to various therapies in vivo. These efforts involve important collaborations with several CSHL investigators and will be an important part of future efforts.

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CANCER INTEGRATIVE GENOMICS: EPIGENETIC ANALYSIS OF CANCER

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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 which are next to guanine residues. It has been shown that methylation present in the DNA of the transcriptional control region has been involved in the silencing of gene expression of tumor suppressors in cancer. We have adapted representational oligonucleotide microarray analysis (ROMA) to methylation detection oligonucleotide microarray analysis (MOMA). The present array queries all CpG islands within the genome. To detect methylation, input DNA (either tumor or normal) is cleaved with MspI and then ligated to polymerase chain reaction (PCR) adaptors and divided into two samples. One sample is digested with the endonuclease McrBC, which recognizes and cleaves methylated DNA with the promiscuous recognition sequence A/GmC(N40-3000)A/GmC (Gast et al., *Biol. Chem. 378:* 975–982 [1997]) depleting methylated sequences. The other sample, the reference, is mock-digested. Both samples are amplified by PCR, differentially labeled, and hybridized to the array.

This methodology was used on cell lines, and 11 of 11 methylation measurements were validated by bisulfite sequencing confirming its accuracy. Although the standard method of methylation profiling of tumors is to utilize matched normals, many tumor banks contains tumor samples without matched normals. Using a set of matched tumor normals (12 normals, 12 tumors) and a set of matched and unmatched tumor normals (12 normals, 28 tumors), we developed statistical criteria for identifying CpG islands most significantly altered between tumor and normal even when unmatched. We looked at all permutations comparing matched and unmatched tumors to all normals and found that all tumors generally varied similarly from the normals whether matched or unmatched. In other words, all tumors are similarly different from normals. We then moved onto the analysis of a larger set of 40 breast tumors, compared to 12 normal breast samples, and 11 ovarian tumors, compared to 7 normal ovarian samples. Using the statistical criteria developed, we obtained a list of 916 significant alterations in breast cancer and 151 in ovarian cancer, many of which have previously been documented to have altered methylation in cancer, again demonstrating the ability of this methodology to detect CpG methylation in cancer. For example, the *MTSS1* (metastasis suppressor 1) gene is known to be preferentially methylated in several cancers including breast cancer, and its lack of expression correlates well with increased Her2 expression. Genes that we identified as being hypermethylated in the ovarian and breast tumors include several HOX genes and protocadherins, which are known to be methylated in many tumor types. In addition to genes known to undergo methylation, we have found new targets of methylation. We also detected for the first time promoter methylation of a microRNA gene, *has-mir-9-3*, occurring in more than half the breast tumors. The expression of this microRNA has been shown to be down-regulated transcriptionally in breast and other cancers.

Studies suggest that the methylation profile can be used to readily distinguish tumor samples from normal samples. To confirm and extend these observations, we performed hierarchical clustering on the 100 most significantly altered CpG islands between breast tumors and normals and ovarian tumors and normals. In all sample sets, the normals segregated from tumors. We also used a supervised machine-learning classifier to identify the sensitivity and specificity of using methylation to differentiate breast tumor samples from normals (the ovarian tumor data set was too small to perform this analysis) and used a "leave-one-out" cross-validation to calculate the sensitivity and specificity. Using only ten CpG islands, we achieved a classification accuracy of 94%. Sensitivity for tumor detection was 92.5% (37/40) and specificity for tumor detection was 100%, demonstrating the potential that tissue can be identified as tumor or normal based on a relatively small number of methylation alterations. In addition, of the breast tumors, a subset has been shown to segregate into six subclasses based on expression analysis data; hierarchical clustering performed on the methylation data recapitulated these subclasses, demonstrating that the methylation data can be used to

stratify tumor samples. We propose that the methylation data can be used to identify biomarkers that stratify with clinical parameters, and we will be studying this in the future.

We compared methylation status between promoter-specific islands (TSS-CGIs) and nonpromoter islands (non-TSS-CGIs), as well as non-island CpG dinucleotides (non-CGIs), because this could be informative with respect to tumor evolution. We found that the methylation events that were frequently found were methylation of specific TSS-CGIs and non-TSS-CGIs and demethylation of non-CGIs. What was interesting was that for the class of non-TSS-CGIs, although we found some islands frequently methylated, the vast majority were demethylated. However, this demethylation was random and rarely were the same islands demethylated in multiple tumors. This greater preponderance of demethylation in cancer without recurrence suggests that the demethylation of non-TSS-CGIs is more of a random event in the genome, compared to the methylation of TSS-CGIs being specific. Perhaps this is a result of the methylation of TSS-CGIs being positively selected during carcinogenesis in order to silence tumor suppressor genes, whereas the demethylation is not being selected for and the majority appears to be random. More than likely, methyltransferases are utilized for methylation, but it is unclear how demethylation occurs within an environment of methylation. It is possible that it is as simple as the methylation process on a whole being deregulated, resulting in some regions being aberrantly methylated and others not being methylated. Because we are looking at the tumor after its evolution, we only see the cells most successful at survival. The methylation of specific genes is selected for and the majority of the other methylation events are not observed because they are unselected. This will be the subject of future studies.

Copy-number Analysis of Cancer

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Mutation of the genome is central to the development and progression of cancer. Mutations occur in the genome of the precancerous cells and accumulate, altering gene function until the growth of these cells goes unchecked. The genes responsible for cancer must be identified if we are to understand the cellular pathways that get subverted and allow the cell to become cancerous. We have developed a genomic microarray technique, ROMA, to identify copy-number fluctuations.

PANCREATIC CANCER

According to the American Cancer Society, there will be an estimated 37,170 cases of pancreatic cancer this year. Of those, 33,370 patients will succumb to the disease. Although the number of cases is low, in comparison to several other cancers such as colon, lung, or breast cancer, the survival rate for pancreatic cancer is one of the lowest. Treatments can extend survival or alleviate pain but seldom cure the patient. In fact, the mean survival time is approximately 6 months. Because the life span after diagnosis is very short, the number of patients that receive tumor resections is very low. This translates to few samples available for analysis. Because of this, we are collaborating with many clinicians to put together a useful tumor bank of pancreatic tissue for analysis. Currently, we are collaborating with Dr. Ralph Hruban of The Johns Hopkins University School of Medicine, Dr. Daniel Von Hoff of the Arizona Cancer Center, and Dr. Vijay Yajnic of the Massachusetts General Hospital, who will be providing pancreatic specimens, clinical information, and expertise. To date, we have analyzed 98 samples. We have confirmed many of the known mutated regions and have identified less-characterized regions.

One region on chromosome 19q12 that is amplified is interesting because there has been confusion in the literature as to which gene in the region is the oncogene with respect to pancreatic cancer. A number of tumors and cell lines have this region amplified, but two primary tumors are the most informative for defining the epicenter or common region of mutation. Previously, we have shown that a gene named *p21*-activated kinase (*PAK4*) was overexpressed at the RNA level, overproduced at the protein level, and overactivated as a kinase in cells with genomic amplification. This protein was also shown by other investigators to transform cells when activated.

To elucidate the function of *PAK4* in the tumor, we have used short hairpin RNA (shRNA) constructs to knock down the level of *PAK4* in cells with the amplicon to determine the effect on tumorigenicity. After knocking down the level of *PAK4* in the cells, we collected RNA and performed expression analysis to determine if there was a change in the cellular environment. Tumors are very heterogeneous, including their expression repertoire. Therefore, we performed this analysis for three separate cell lines with this region genomically

amplified and used the results from all three to filter out noise and determine which were in common. Of the genes found with expression affected by *PAK4*, one interesting candidate is DUSP22 (JSP1), a phosphatase that is involved in selective activation of the mitogen-activated protein kinase (MAPK)/Jnk pathway, a pathway described below important for cell survival.

The region with *PAK4* amplification is found amplified in several cancers, including pancreas, breast, and ovarian. Of these cancers, activity in breast cancer was tested, and we have determined that its activity is increased. Because *PAK4* is found amplified in several tumor types, we have generated a transgenic mouse with *PAK4* overexpressed in all tissues. The mice are 3 months old and, thus far, are phenotypically normal. We plan to cross these mice to mice with other genes such as activated K-*ras*, a gene known to activate *PAK4* and frequently activated in pancreatic cancer. Due to the construction of the K-*ras* mice, the K-*ras* gene is turned on only in specific tissue and thus we can test the role of *PAK4* in specific organs such as pancreas, breast, or ovaries.

OVARIAN CANCER

We are also focusing on the analysis of ovarian cancer using ROMA. Ovarian cancer has a relatively high incidence and approximately 50% survival rate. In many patients, the cancer is diagnosed late, often having metastasized, the first symptoms being an accumulation of fluid in the abdominal cavity. There have been few genes discovered that are involved in the progression of ovarian cancer. We will collaborate with Dr. Michael Pearl of SUNY Stony Brook for access to tissue and clinical information. We will also perform ROMA on a tumor set of approximately 200 ovarian cancer samples to identify gene-copy-number fluctuations to identify candidate tumor suppressors and oncogenes.

At present, we have analyzed 121 tumors and have compiled the data to identify regions commonly amplified or deleted. One such uncharacterized region we are studying is a deletion on chromosome 5q14. This region contains seven genes. Of the tumors analyzed, we had copy number and expression and now have methylation data. For all candidates, the three data types were integrated. In addition, available published expression data were also associated with the data (in this case, an ovarian expression data set was available). It was found that of the seven genes deleted, one gene was also methylated and expression was repressed, including being repressed in the available public expression data. This gene is *p21* RasGAP. The protein activates the intrinsic GTPase acitivity of *ras* and once Ras is bound to GDP, it is no longer active. Therefore, it essentially inhibits Ras. Our hypothesis is that methylation represses transcription of this inhibitor and thus leaves Ras in an active state longer. We are presently knocking out RasGAP in cells that express it at normal levels to determine the advantage to the tumor cell.

Mutational Analysis of Phosphatases

E. Lum, T. Auletta, [in collaboration with N. Tonks and S. Muthuswamy, 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.

Previously, we used genomic-copy-number data to identify the phosphatases, LAR, PTPsigma, and VHY gene-amplified in ovarian tumor specimens. LAR and PTPsigma are receptor-like PTPs, display features of cell-adhesion molecules, and have been implicated in regulating the function of adhesion complexes that control cell–cell interactions and may be disrupted in cancer. VHY is a dual specificity phosphatase that the Tonks' lab has implicated in the activation of a signaling pathway (the JNK pathway), which has a critical role in the control of cell survival. MKP5 is another dual specificity phosphatase that functions in the inactivation of the p38 and JNK MAPKs.

Oncogenes and tumor suppressor genes are frequently altered in their expression between normal and tumor specimens. Expression data from cell lines were used to determine if any of the phosphatases were aberrantly expressed and to cross-check against the copy number and methylation data. Of the phosphatase genes found gene amplified, PTPsigma and LAR showed the most significant expression changes, making them the best candidates. In addition to our analysis of phosphatases that have undergone alterations with gene copy number, we conducted transcriptional expression analysis on the remaining phosphatases. Two sets of expression data were used to remove genes found in only one set; 15 phosphatases showed overexpression and/or underexpression in both sets. Of these, 3 overlapping phosphatases were found to show significant change, including DUSP6, PTPRK, and PTPRZ1; these will be investigated further in the upcoming year.

Interestingly, a binding partner for the receptor PTP LAR, Liprin, is highly amplified and displays altered gene expression. Liprin was shown originally to be important for targeting LAR to focal adhesions and for clustering the PTP at these sites, regulating cytoskeletal function and interactions with extracellular matrix. This observation not only highlights the potential significance of LAR in ovarian cancer, but also, and more generally, introduces an interesting extension of the primary project, i.e., an analysis of the proteins that interact with the PTPs. The importance of genes that interact with phosphatases is further highlighted by the *MTSS1* gene that was found methylated. The MTSS1 protein interacts with RTPdelta, a receptor-like PTP, and cooperates in cytoskeletal reorganization. We are currently collaborating with Dr. Tonks and Dr. Muthuswamy to determine the role of *MTSS1* in tumorigenesis.

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Chris Johns

James Byrnes

MAMMALIAN FUNCTIONAL GENOMICS

A. Mills N. Alston W. Li
A. Bagchi C. Papazoglu
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W. Keyes Y. Wu

The Mills lab (Fig. 1) designs and utilizes novel models for elucidating the genetic and molecular basis of human disease. Research areas include:

1. Determining the role of the chromatin remodeler CHD5 in cancer and stem cell function.
2. Investigating of the role of the p53-related protein p63 in development, cancer, and aging.

CHD5 IS A TUMOR SUPPRESSOR MAPPING TO *1P36*

Deletions encompassing *1p36*, a portion of the genome residing on human chromosome 1, were first reported in 1977. Numerous studies of late-stage tumors have indicated that *1p36* is frequently deleted in a variety of human cancers, including those of the brain and blood, as well as epithelial malignancies such as those of the breast, colon, and prostate. Although this suggests that an important cancer-preventing gene resides at *1p36,* this tumor suppressor gene had not been identified. Within the past year, we identified Chd5 as a novel tumor suppressor. We used chromosome engineering to generate models with gain and loss of the region of the genome corresponding to human *1p36* and studied these models for predisposition to or protection from cancer.

Chromosome engineering combines the power of gene targeting with Cre/*loxp* technology and allows the generation of mouse strains that harbor precise rearrangements such as deletions and duplications; these models have loss and gain of specific regions of the genome, respectively. This strategy allowed us to identify a 4.3-Mb interval with potent tumor suppressive activity. Whereas loss of this region caused a marked increase in cellular proliferation that culminated in cancer in vivo, gain of the same interval triggered excessive tumor suppression as observed by augmented cellular senescence in cultured cells and apoptosis in vivo. We used a combination of genetic and molecular analyses to pinpoint the chromatin remodeling protein Chromodomain, helicase, DNA-binding domain protein 5 (*Chd5*) as the tumor suppressor in the interval. In addition, we demonstrated that Chd5 functions as a master regulator that facilitates p16/Rb- and p19/p53-modulated pathways. Analyses of human gliomas revealed that *Chd5* is frequently deleted, suggesting that Chd5 loss contributes to this particular neural malignancy. Mice heterozygous for the region encompassing *Chd5* are currently being monitored for spontaneous tumors. In addition, we are investigating the role of Chd5 in stem cells. This work identifies a novel tumor suppressor, paving the way for designing more effective anticancer therapies for a wide variety of human cancers.

FUNCTION OF THE *P53* HOMOLOG *P63*

p63 deficiency is tumor-protective. The discovery that the *p53* tumor suppressor is a member of a multigene family that also includes *p63* and *p73* has brought the p53 field into a new era. p63 is a transcription factor structurally and functionally similar to p53; in contrast to p53, however, p63 is rarely inactivated in human cancers. In fact, the *3q27* region to which *p63* maps is frequently amplified, or p63 is overexpressed in the majority of epithelial tumors, suggesting that p63 has oncogenic potential. We previously identified *p63* and generated a number of p63-deficient mouse models. What was clear from the phenotype was that despite the striking similarities between p63 and p53, they perform very different functional roles in vivo: p63 is essential for development of stratified epithelia, whereas p53 is dispensable during embryogenesis but functions as a potent tumor suppressor in the adult. More recently, we found that in contrast to the high incidence of spontaneous tumors in $p53^{+/-}$ mice, $p63^{+/-}$ mice were not predisposed to cancer. In fact, *p63* heterozygosity *decreased* the high tumor incidence of $p53^{+/-}$ mice, indicating that haploid levels of p63 may even be tumor-protective. Loss of the wild-type *p63* allele did not occur and p63 expression was maintained in the rare tumors that did develop in the $p63^{+/-}$ cohort. $p63^{+/-}$ mice were not even susceptible to chemical carcinogens. This indicated that p63 does not perform a role equivalent to p53 in tumor suppression and that reduced p63 may provide a novel tumor suppressive mechanism, suggesting that modulation of p63-mediated pathways could offer an effective strategy for anticancer therapy. We are cur-

rently working on determining which of the six different p63 proteins modulate the tumor-suppressive mechanism of cellular senescence.

p63 links cellular senescence and aging. Over the course of the tumor study outlined above, we discovered that $p63^{+/-}$ mice had a significant reduction in life span and developed age-related pathology. Using a conditional system to shut down p63 function specifically in proliferating cells of stratified epithelia such as the skin, we discovered an unanticipated link among p63, cellular senescence, and aging. Remarkably, p63 deficiency activates a program of cellular senescence and leads to accelerated aging. This finding suggests a mechanism for the low tumor incidence of $p63^{+/-}$ mice: Cellular senescence effectively removes aberrantly proliferating cells from the proliferative pool, thus providing a tumor-suppressive mechanism. A further understanding of how p63-mediated pathways modulate stem cells and how this affects aging and tumorigenesis is currently under way. This work will provide a clearer understanding of the mechanism of cellular senescence that will ultimately impact our understanding of tumorigenesis and stem cell homeostasis during aging.

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The Mills Laboratory: (*Top row*) Anindya Bagchi, Bill Keyes, Assaf Vestin, Cristian Papazoglu, Ying Wu; (*bottom row*) Baoyi Li, Mathangi Ramesh, Ninche Alston, Alea Mills, Xuecui Guo. (*Not shown*: Shilpi Paul.)

CANCER GENES

S. Powers C. Cai D. Mu
Q. Liu M. Rajaram
N. Liu-Sullivan J. Zhang

A growing trend in cancer research is to focus less on pure basic research and more on transforming laboratory discoveries into clinical applications. Translational research, which includes the discovery and characterization of promising molecules or gene targets for cancer therapeutics, also involves establishing collaborations with clinicians and industry so that we can move basic laboratory discoveries forward into real applications. Highlighted below are our translational research efforts in 2007.

A Functional Genetic Screen Leads to a Potential New Combination Therapy

N. Liu-Sullivan, J. Zhang, S. Powers [in collaboration with G. Hannon, Cold Spring Harbor Laboratory, and GlaxoSmithKline]

In late 2006, we joined a consortium of academic investigators who are funded by the pharmaceutical company GlaxoSmithKline (GSK) to propel the clinical development of their candidate cancer drug portfolio. There are two ways in which academic labs can help move late-stage drug candidates forward into clinical use. One is to discover a test that pinpoints which patients will respond to the treatment. The other way is to discover drugs that synergize with and significantly increase the efficacy of the candidate drug.

Our project has focused on GSK's polo-like kinase inhibitor. The polo-like kinase gene (*PLK1*) is frequently overexpressed in cancer and is associated with higher proliferation and poor prognosis. Polo-like kinase regulates several key events during mitotic progression, and its inhibition selectively kills cancer cells for reasons that are not completely understood. To look for genes that influence the response of cancer cells to the polo-kinase inhibitor, we performed a functional genetic screen targeting 1500 cancer-relevant genes with a microRNA-based short hairpin RNA (shRNA)-focused library. We found that silencing of the retinoic acid receptor α gene (*RARA*) conferred resistance to polo-kinase inhibition. Stimulation of this receptor by its natural ligand and approved cancer drug retinoic acid (ATRA) had the opposite effect of sensitizing cancer cells to polo-kinase inhibition. We are currently working with GSK to determine whether these results hold true in animal models and whether to move forward into clinical trials with this combination therapy.

Gene Targets for New Cancer Therapeutics in Liver Cancer

C. Cai, J. Zhang, Q. Liu, S. Powers [in collaboration with S. Lowe, Cold Spring Harbor Laboratory]

The clinical success of Gleevec and Herceptin, which target the mutated cancer genes *BCR-ABL* and *HER2*, respectively, provided proof-of-principle that mutated cancer genes are appropriate targets for cancer therapeutics. There is a shortage of such targets for liver cancer and our laboratory is currently receiving funding from the National Institutes of Health (NIH) to find cancer gene targets for this cancer type. As part of this effort, in early 2007, we integrated the large RNA expression and DNA copy-number liver cancer data sets that we had accumulated to perform a genome-wide determination of genes that are overexpressed as a result of gene amplification.

We discovered that the most common amplicon in liver cancer, the well-known 11q13 amplicon containing *CCND1* (the cyclin D1 oncogene discovered by David Beach at CSHL in 1991), contains an additional candidate driver gene, *FGF19*. *FGF19* encodes a secreted growth factor and this type of protein can be easily inhibited by monoclonal antibodies. We were therefore very pleased when we found that *FGF19* is indeed a functional oncogene in liver cells. It is likely that *FGF19* was overlooked by previous investigators because they were studying tumor types in which *FGF19* is not turned on by gene amplification. We are currently exploring the generality of *FGF19* oncogene dependence in human liver cancer cells, which will largely define how promising this is as a gene target for a new liver cancer therapeutic.

We have also begun a systematic functional screening for the oncogenicity of amplified genes in liver can-

cer. To do this, we have leveraged the NIH-funded genome resource of sequence-verified human cDNA expression vectors (the Mammalian Gene Collection). From a select group of genes that are highly amplified in human liver cancer samples, we have transferred 108 cDNAs into retroviral expression vectors. These vectors were used to test effects on tumor formation in animals, and our results indicate that 17 of the 108 genes are capable of driving cancer progression in liver cells. Of these 17 new amplified oncogenes, 2 are capable of being inhibited by antibodies. We are now focusing our attention on determining whether these two genes are promising targets for monoclonal antibody therapy.

Discovery of a Major New Genetic Alteration in Lung Cancer

D. Mu, Q. Liu, S. Powers [in collaboration with J. Kendall in M. Wigler's lab, Cold Spring Harbor Laboratory; W. Gerard, Memorial Sloan-Kettering Cancer Center; and A. Potti, Duke University]

This year, we discovered a novel genetic defect that affects 20% of lung cancers. This gene-amplification defect affects three genes that are located within a 150-kb segment on chromosome 14 and results in increased expression of the three transcription factors (*TTF1, NKX2-8*, and *PAX9*). Both *TTF1* and *NKX2-8* have fundamental roles in fetal lung development, and *PAX9* is also a developmental transcription factor. We performed functional studies to show that *TTF1, NKX2-8*, and *PAX9* cooperate to speed up the growth rate of lung cells, implying that the 20% of lung tumors with this novel mutation rely upon the synergistic interaction of these developmental genes for rapid growth (see Fig. 1). We also showed that the affected lung tumors likely remain addicted to each of the amplified genes using RNA interference (RNAi). In collaboration with Dr. William Gerald at Sloan-Kettering, we found that amplification of this region is associated with late-stage lung tumors and is possibly a risk factor for recurrence.

We hypothesize that certain lung cancers use gene amplification of this region to reactivate a specific fetal lung development process. We performed transcriptome analysis to determine whether the global effects of these amplified transcription factor genes could be used to uncover clinically useful subtypes of human lung cancer. In collaboration with Dr. Anil Potti at Duke University, we found that gene expression patterns corresponding to different combinations of these transcription factor oncogenes can predict both sensitivity and resistance to cisplatin—the standard treatment for advanced lung cancer—with much greater accuracy than any predictor, including clinical parameters, previous genomic predictors, and *P53* and K-*RAS* status. This exciting result provides a potential new diagnostic test to guide treatment in lung cancer.

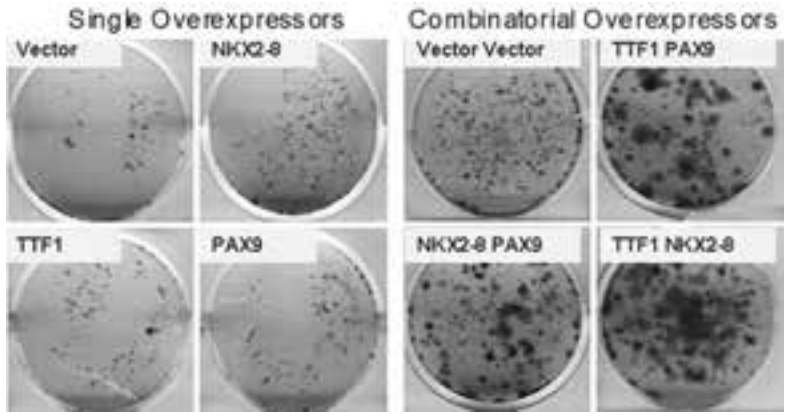

FIGURE 1 Demonstration of oncogenic cooperation between the three genes within the commonly amplified region of the 14q13 lung cancer amplicon. The recipient cell was a nontumorigenic SV40-immortalized human lung epithelial cell. Derivatives expressing different combinations of the three genes were derived by retroviral transfection, and the effect on growth was assayed by colony formation of cells plated at low density. Single overexpressors of any of the three genes were without significant effect, but all pairwise combinations showed significant growth-promoting effects.

Genomic Identification of New Therapeutic Targets and Diagnostics in Melanoma

S. Powers [in collaboration with R. Lucito, Cold Spring Harbor Laboratory; A. Houghton, N. Rosen, G. Schwartz, A. Halpern, and J. Wolchok, Memorial Sloan-Kettering Cancer Center]

In late 2006, we established a collaboration with an accomplished group of clinician-scientists studying melanoma at Memorial Sloan-Kettering Cancer Center. Our work in this collaboration is enabled by grants from the Lita Annenberg Hazen Foundation and more recently from the Starr Consortium. During the last year, we built up a large data set of DNA copy-number alterations in melanoma and have several amplified oncogene candidates that we are evaluating for their suitability as therapeutic targets. Our second approach has been to use RNAi screening to look for genetic vulnerabilities in different classes of melanoma. This is a complementary approach for target identification. Rather than aiming to counteract the DNA alterations that drive melanoma formation, we are conducting an unbiased search for genetic vulnerabilities in cancer cells.

Most Focal Deletions in Colorectal Cancer Are Secondary Consequences of Cancer Progression

M. Rajaram, D. Mu, J. Zhang, Q. Liu, S. Powers

A basic premise of cancer research is that genetic alterations that occur in many tumors—such as loss of *TP53* or point mutational activation of K-*RAS*—provide an important selective advantage to evolving tumor cells. However, this year, we found that this is not necessarily so, particularly for homozygous and other focal deletions.

When we started our laboratory in 2004, we began a comprehensive analysis of DNA copy-number alterations in colon cancer genomes using high-resolution ROMA (representational oligonucleotide microarray analysis) arrays. As noted in prior annual reports, we initially believed that the numerous focal deletions which we had discovered contained a treasure trove of tumor suppressor candidates. We have now completed a systematic analysis of the biological significance of focal deletions in colon cancer on a genome-wide scale. We found that they occur independently of the two well-established forms of genetic instabilities in colon cancers: chromosomal instability and the microsatellite instability caused by mismatch repair defects. Instead, the sites most frequently deleted closely match common sites of chromosomal breaks caused by replicative stress.

We are now testing the hypothesis that the underlying cause of these focal deletions is a distinct form of genetic instability that results from replicative stress. A prediction of this hypothesis is that the frequency of recurrence does not need to reflect biological selection, since its frequency is driven by the susceptibility of the locus to replicative stress-induced breakage. In support of this prediction, we found numerous distinctions between recurrent focal deletions in colon cancer compared to those affecting the well-established tumor suppressor gene *p16/INK4A* in melanomas, and all of these distinctions argue against a role for these focal deletions in providing a selective advantage during colon cancer progression. For example, deletions in colon cancer are often within introns and have no effect on the expression of the underlying gene. Although we found rare examples of focal deletions affecting bona fide tumor suppressor genes (e.g., *SMAD4*), our analysis leads to the sobering conclusion that most recurrent focal deletions are inactive vestiges of cancer genome instability. This doesn't appear to be the case for all cancers; neither liver cancer nor melanoma exhibit such deletions.

Development of a Web Site for Mining CSHL ROMA Data Sets

J. Zhang, S. Powers [in collaboration with A. Leotta, M. Wigler's lab, Cold Spring Harbor Laboratory; W. Zhu, Stony Brook University]

To help scientists at the CSHL Cancer Center take full advantage of the substantial data sets of DNA copy-number alterations in breast, colon, lung, melanoma, liver, pancreatic, prostate, and other cancers that have been generated here using ROMA during the past 3 years, we created an internal Web site—backed by a dynamic database—that allows cancer biologists to explore the amplification or deletion status of any gene in a panel of more than 700 tumors. We also integrated RNA expression data sets, and in the next year, we will begin to incorporate the increasingly large amount of cancer genome copy-number profiles that are being generated by external groups. Our long-term goal for this Web site is to integrate all genome-wide cancer analyses (including mutational and methylation status) to provide a simple, comprehensive platform to evaluate a gene of interest for its potential involvement in human cancer.

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Baoyi Liu

COPY-NUMBER ANALYSIS AND HUMAN DISEASE

M. Wigler G. Alex, C. Algieri, S. Chakraborty, K. Cook, D. Esposito, V. Grubor, J. Hicks, L. Hufnagel, R. Kandasamy, J. Kendall, A. Krasnitz, Y.-H. Lee, A. Leotta, D. Levy, S. Marks, J. Meth, L. Muthuswamy, N. Navin, D. Pai, M. Riggs, L. Rodgers, M. Ronemus, J. Troge, B. Yamrom

MAMMALIAN GENETICS

We study variations in the human genome that arise when a large segment of the genome is duplicated or deleted. Such copy-number variations, or CNVs, can arise somatically or in the germ line. The former are often seen in cancer, distinguish cancers from the normal cells of the body, and provide clues for the origin and behavior of the cancers. The latter distinguish individuals from each other and may be inherited, in which case, they are known as copy-number polymorphism, or CNPs, or they may arise spontaneously, in which case they serve as engines of human diversity, and can cause devastating genetic disorders, such as autism.

Our studies to date are largely based on a high-throughput high-resolution microarray technology developed at CSHL called ROMA (representational oligonucleotide microarray analysis), which itself was based on an earlier technology developed at CSHL called RDA (representational difference analysis) that was used for discovering tumor suppressors, oncogenes, and pathogens. ROMA is a form of a more general technology called CGH, or comparative genome hybridization. CGH is evolving, and part of our laboratory works on technical improvements and extensions, such as the use of a derivative technique called MOMA (methylation detection oligonucleotide microarray analysis) to study DNA methylation, and on more powerful statistical methods for data interpretation. Part of the lab uses copy-number data and DNA methylation status to study solid cancers, especially breast cancer and B-cell chronic leukemia (B-CLL). We seek to identify the genes most frequently mutated in cancers and leukemias and, in general, to determine whether genomic data can be used to predict the outcome of the disease and its response to therapy. Finally, part of the lab studies CNVs that may underlie autism, congenital heart defects, and other profound disorders of normal human development.

CANCER AND LEUKEMIA

In this past year, our studies on both breast cancer and leukemia have moved from the discovery phase toward clinical trials that will directly affect patient care. Our work to date indicates the superiority of CGH to the traditional clinical standard of genome analysis: fluorescent in situ hybridization (FISH). In the breast cancer area, we are participating in the evaluation of a clinical trial initiated at the Radium Hospital in Oslo, Norway that is designed to understand the role of bone marrow micrometastasis in the clinical outcome of breast cancer. The evaluation of copy-number data with clinical parameters in this trial is still in progress. We have also enrolled as collaborators with Memorial Sloan-Kettering Cancer Center (MSKCC) and Yale University in the retrospective molecular genomic analysis of cases from previous clinical trials designed to discover better markers for successful treatment with chemotherapeutic drugs.

We continue to focus on samples from two Scandinavian collaborations, with Anders Zetterberg at the Karolinska Institute, Sweden, and Anne-Lisa Borresen-Dale at the Radium Hospital, Norway, and with Larry Norton at MSKCC, New York. These studies have elucidated a set of loci, called epicenters, that are the recurrent sites for genome amplification and deletion in breast cancer. The set of breast cancer epicenters overlap with epicenters from lung cancer (data from Scott Powers and David Mu, CSHL) but are clearly a distinct set. In fact, we can distinguish breast cancers from lung cancers largely by the loci involved in amplification and deletion, a method that may be useful in a clinical setting. The epicenters are locations where many of the genes that drive the progression of malignancy reside, and hence, the elucidation of these loci may facilitate drug design and the production of focused and less-expensive tools for the evaluation of the clinical state of cancers.

We have begun a series of studies on tumor heterogeneity. Clues to the progression of cancer, and its clinical profile, are missed if we assume that each individual's cancer is a single clone with a single molecular history. Work in progress indicates that at least half of breast cancers are composed of multiple clones, proves that sampling one region of the cancer for molecular markers yields an incomplete picture, and provides

clues about the origins of breast cancer: the stages through which it passes and the speed with which it evolves.

Our studies of B-cell leukemia, a collaboration with Nick Chiorazzi of North Shore University Hospital, Manhasset, New York, uses ROMA to identify essentially all the known recurrent lesions that have been observed in that disease, as well as several new epicenters. We are in the process of designing B-CLL "tiling" arrays that will allow us to examine the leukemic epicenters at greater resolution, so that we will be able to narrow the gene candidates in each region and assess their recurrence with greater accuracy. Such a B-CLL chip may enable oncologists to rapidly assess the progression of the disease and guide decisions about therapy. As with breast cancer, we now have evidence of multiple clonogenic histories of leukemia in the same patient.

We have made significant progress in our studies of DNA methylation in cancer. Last year, we described results achieved, in collaboration with Rob Lucito here at CSHL, on a microarray platform (MOMA) designed to detect methylation of CpG islands. Now, we have identified genes that change methylation state during the initiation and progress of breast tumors. In our first efforts at determining the clinical significance of methylation, we have analyzed approximately 80 tumors from a clinical trial initiated at the Radium Hospital in Oslo, Norway, that is designed to understand the role of bone marrow micrometastasis in the clinical outcome of breast cancer. The results of methylation microarray analysis on this collection of patient samples along with a separate collection of breast tumors and adjacent normal breast tissue show that the methylation patterns clearly differ between normal tissue and tumor tissue. More surprising, however, is the result that the tumor DNAs naturally cluster into subgroups using methylation data alone. These subgroups match the clustering determined by gene expression. These studies will help us to understand the role of methylation in cancer etiology and facilitate clinical subtyping.

GENETIC DISORDERS

Since our seminal discovery that CNV is common in the human gene pool, we have studied the role of CNVs in human disease and, in particular, the role of spontaneous or de novo CNVs in autism. In a collaboration that continued with Jonathan Sebat here at cSHL, we tested the hypothesis that de novo CNV is associated with autism spectrum disorders (ASD). We performed CGH on the DNA of patients and unaffected subjects to detect copy-number variants not present in their respective parents. Candidate genomic regions were validated by higher-resolution CGH, paternity testing, cytogenetics, FISH, and microsatellite genotyping. Confirmed de novo CNVs were significantly associated with autism. Such CNVs were identified in about 10% of patients with sporadic autism, in about 2% of patients with an affected first-degree relative, and in only about 1% of controls. Most de novo CNVs were smaller than microscopic resolution. Affected genomic regions were highly heterogeneous and included mutations of single genes. These findings establish de novo germ-line mutation as a more significant risk factor for ASD than previously recognized and clearly point to a new approach for the further study of the genetic basis of this and other genetic disorders, such as schizophrenia and congenital heart disease.

Consonant with the finding of spontaneous CNVs in humans, studies in mice, a collaboration with Ira Hall here at CSHL, have demonstrated that de novo CNVs occur frequently in mice lineages. These studies indicated that certain loci are vastly more unstable than others and is consonant with the observation that many of the de novo changes in humans are recurrent.

Additional analysis of autism incidence data, a collaboration with Kenny Ye at Albert Einstein School of Medicine, has provided evidence for an important genetic model for the disorder. Autism families are divided into simplex (only one affected child) and multiplex (multiply affected children). In the latter case, we found by inspecting the records from the AGRE consortium that the risk to a male newborn in an established multiplex family is nearly 50%, the frequency expected of a dominant disorder. Our analysis of polymorphism data virtually rules out that this can be explained as inheritance on the X-chromosome. We find that overall autism incidence is consistent with a model in which new mutations arise that have a strong contribution to the disorder and are passed from a resistant carrier parent to a child in dominant fashion. One major source of resistance is in fact gender, and a singularly important fact about autism is its lower incidence in females.

We are now in the midst of preparing for a much larger-scale study of spontaneous mutation in simplex autism, dependent on a population of families being organized by the Simons Foundation. Hopefully, this 3-year study will lead to the identification of recurrent loci that contribute to the majority of autism risk and might thereby elucidate convergent pathways involved in the etiology of the disorder, possibly leading to treatments in some cases. One clear benefit of such a large study will be in the design of clinical tests for genetic coun-

seling, early detection, and perhaps matching patients with the most effective therapies.

DATA GENERATION AND ANALYSIS

The major part of our group's effort centers on the generation, analysis, and organization of data. This entails developing protocols for conducting microarray experiments; determining quality control, probe evaluation, signal extraction, and segmentation (the method of "observing" CNV); comparisons of sets of experiments, including new statistical measures, data reduction, and data summary; and construction of databases so that we can communicate our results to other investigators. Although we usually do not report our methods in this forum, we are in the midst of preparing several manuscripts in which our new statistical, mathematical, and computational ideas have a prominent role. Hopefully, we will detail them in next year's report.

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Alex Garrick

CANCER: SIGNAL TRANSDUCTION

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

Nicholas Tonks and colleagues study a family of enzymes called protein tyrosine phosphatases, or PTPs, which have the beguilingly simple function of removing groups of phosphate molecules from other proteins. PTPs are the "subtractive" half of a fundamental cell regulatory process called reversible phosphorylation. The opposite function—the adding of phosphate groups—is performed by enzymes called kinases. The coordinated and competing actions of kinases and phosphatases control signaling output in cells. By changing the phosphorylation state of proteins, PTPs initiate cascades that can profoundly affect the health of entire organisms. Tonks' group seeks to fully characterize the PTP enzyme superfamily, understand how their activity modifies signaling pathways, and how those pathways are abrogated in serious illnesses, from cancer to Parkinson's disease.

Linda Van Aelst's lab is studying how aberrations in intracellular signaling involving enzymes called small GTPases can result in disease. They are particularly interested in Ras and Rho GTPases, which have roles in controlling cellular growth, differentiation, and morphogenesis. Alterations affecting Ras and Rho function are involved in several diseases, including cancer and various neurodevelopmental disorders. This year, Van Aelst's team extended their study of mutations in a Rho-linked gene called *oligophrenin-1*, determining that it is involved in the morphogenesis of dendritic spines, the main site of excitatory nerve synapses. They are probing the implications for several illnesses. The lab also demonstrated that a GTPase activator called DOCK7, previously shown to have a central role in axonal development and morphology, is important in neuronal migration and may be involved in developmental pathology.

Yuri Lazebnik and his team are exploring the hypothesis that viruses and other common human pathogens might be capable of causing cancer under certain conditions. They recently completed a study demonstrating that massive chromosomal instability could be engendered by a transient event causing destabilization of the genome without permanently affecting mechanisms such as mitosis or proliferation. The agent, in this instance, was an otherwise harmless virus that caused chromosomal disruption by fusing cells whose cell cycle was deregulated by oncogenes. The resulting cells had unique sets of chromosomes, or karyotypes, and some proved to be capable of producing aggressive epithelial cancers in mice.

Senthil Muthuswamy regards the regulation of cell architecture as a driving mechanism in cancer. His lab is studying cell systems, in culture and in vivo, to understand the relation between proliferation and morphogenesis. In breast cancer, for instance, the morphogenic program seems to be disturbed. The lab studies genes that regulate and maintain cell shape and has demonstrated that specific oncogenes, in addition to inducing proliferation and inhibiting programmed cell death, "talk" via signaling pathways to machineries involved in the regulation of cell morphology. The regulatory process goes awry, resulting in cell structures manifesting dysfunctional morphological orientations, or "polarities," an early step, perhaps, in the genesis of cancerous lesions. Muthuswamy and colleagues are studying human epithelial cells with the aim of understanding cancer-specific changes in polarity pathways and identifying potential therapeutic strategies for epithelial and other cancer types.

Jacek Skowronski and colleagues study mechanisms involved in the induction of AIDS by human and simian immunodeficiency viruses (HIV and SIV), focusing on the function of accessory proteins called Nef, Vpr, and Vpx. These are important virulence factors, which modify the cellular milieu to disrupt adaptive and/or innate antiviral responses and provide an environment conducive for viral replication. This year, Skowronski's team explained how Vpr proteins in HIV and SIV overcome restrictions that host cells impose on their reproduction, by associating with a protein complex that in turn perturbs the cell cycle and DNA metabolism in infected cells. By providing a molecular framework for understanding virulence factors such as Vpr, Skowronski hopes to augment efforts to rationally design drugs to control HIV-1 infection in humans.

Despite their large variety of genetic abnormalities, cancer cells have been found to be extremely sensitive to the reversal of certain mutations. The research of Raffaella Sordella is focused on the question of why cells in certain cancers are responsive to the inhibition of one particular gene or gene product. Why, for instance, do non-small-cell lung cancer cells that have a particular epidermal growth factor (EGF) receptor mutation respond so dramatically to the drug Iressa? Answers are likely to bolster the search for new targeted therapies and contribute to a better understanding of how cancer cells eventually develop resistance to them. The Sordella lab is also interested in understanding the etiology of tumors to which EGF receptor mutations give rise, which occur more frequently in women and people of Asian descent.

Lloyd Trotman is examining genetic mechanisms involved in tumor suppression and tumor initiation in mouse models. Trotman proceeds from his discovery several years ago that loss of a single copy of a master tumor-suppressing gene called *PTEN* is sufficient to permit tumorigenesis in animal models of prostate cancer. This disproved the common assumption that cancer could develop only if both copies of a potent suppressor gene were lost. Paradoxically, Trotman and colleagues found that complete loss of *PTEN* triggered senescence, a quiescent state that had the effect of delaying or blocking cancer in affected cells. Currently, the lab is exploring the impact of varying *PTEN* expression levels, alone and in conjunction with changes in other potent tumor suppressors including p53 in mouse models for prostate cancer.

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

Y. Lazebnik D. Duelli
A. Gottesman

Genomic instability (GIN), which is defined as an abnormal and persistent change of a genome over time, is a common feature of many solid cancers and is thought to underlie malignant properties of these tumors. In the majority of solid cancers, GIN is manifested by chromosomal instability (CIN), a condition in which the structure of chromosomes or their assortment changes over time, making the affected cells aneuploid.

It is well documented that the maintenance of CIN is enabled by deficiencies in proteins that police genome integrity, such as the tumor suppressor p53, but the cause(s) of CIN in sporadic cancers remains uncertain. The primary suspects are DNA damage, or mutations that deregulate telomere maintenance or mitosis, yet such mutations have not been identified in the majority of sporadic cancers.

We previously proposed a hypothesis that viruses can contribute to carcinogenesis and tumor progression by fusing cells. The consequences of this fusion could be twofold: combining properties of cells and causing chromosmal instability. During the previous year, we demonstrated that fusing human cells which had relatively stable genomes produced hybrids affected by massive chromosomal instability, as indicated by the observation that each analyzed cell has a unique karyotype. Some of these hybrids, but not the parental cells, produced aggressive, highly aneuploid, heterogeneous, and transplantable cancers in mice.

Because many human viruses are fusogenic, we suggested that viruses, including those that have not been linked to carcinogenesis, contribute to cancer by causing chromosomal instability through cell fusion. We also proposed that detecting causes of cell fusion in solid cancers might lead to viruses that directly or indirectly participate in carcinogenesis. Last year, we began to develop experimental systems to test several aspects of the hypothesis that cell fusion caused by viruses contributes to cancer. In the middle of the year, Dominik Duelli departed to set up his own laboratory at Rosalind Franklin University, and Amy Gottesman and I continued the study.

We previously found that fusion of normal human cells causes cell cycle arrest, which was abrogated by the adenoviral oncogene E1A or by ectopic expression of mutated tumor suppressor p53. We postulated that this arrest was caused by a mechanism that we named the fusion checkpoint (FCP) and suggested that deregulation of the FCP by oncogenes could contribute to carcinogenesis. During 2007, we set up an experimental system to study what the mechanism of this arrest is and how it is regulated. A particular difficulty that we managed to overcome was to develop an approach to analyze cell cycle distribution of individual nuclei in heterokaryons and to distinguish heterokaryons from the cells that became binuclear by failing to divide.

We also focused on investigating how cell fusion causes chromosomal instability by considering two hypotheses. One hypothesis is that CIN is a result of tetraploidy, a natural consequence of cell fusion that might overwhelm the mitotic machinery, thus decreasing fidelity of chromosome segregation. Another hypothesis is that cell fusion causes massive DNA breaks through a process known as premature chromosome condensation, or PCC. To distinguish between these hypotheses, we set up a system in which cells can be either fused or made tetraploid by inhibiting cytokinesis.

One caveat of our study that identified carcinogenic hybrids was that we caused cell fusion by an infectious retrovirus. Although we detected no carcinogenic effect of the virus in a setting in which cell fusion was avoided, the use of an infectious retrovirus left open the possibility that the carcinogenic effect that we observed was due to effects of retroviral infection, such as insertional mutagenesis, that are unrelated to cell fusion. Therefore, we began to develop an experimental system in which cell fusion is caused by ectopically expressed viral fusogenic proteins. This system will also be useful in testing whether cell fusion can affect tumor progression.

Overall, our work during this year has been primarily focused on developing experimental systems that we will use to determine how cell fusion causes cell cycle arrest and how abrogation of this arrest by oncogenic events leads to chromosomal instability. Irrespective of whether any consequences of cell fusion contribute to carcinogenesis, our research will develop an experimental framework to test the role of chromosomal instability in cancer.

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Amy Gottesman

EPITHELIAL CELL BIOLOGY AND CANCER

S. Muthuswamy V. Aranda, K. Bergami, S. Chatterjee, A. Lucs, M. Nolan, A. Rosenberg, L. Zhan

Breast cancer is a leading cause of cancer death among women. Current therapeutic strategies are mostly aimed at controlling malignant breast cancers. Although these strategies are helping patients by extending their life span, they do not stop the cancer. We hope to develop novel strategies that can control cancer better. Our research has two broadly focused goals: (1) to develop novel strategies to diagnose and treat cancer early (precancers) and (2) to identify strategies to enhance therapeutic response of drugs that are currently used in the clinic.

NOVEL STRATEGIES TO TARGET PRECANCER

Increased rates of cell proliferation and disruption of normal tissue architecture are the key changes observed in premalignant lesions. Disruption of tissue architecture is a key criterion used by pathologists to diagnose and grade cancer, supporting a notion that pathways that control tissue architecture have critical roles during cancer initiation and progression. Although pathways that lead to an increase in cell number have been intensely interrogated, very little effort is being directed toward identifying pathways that mediate disruption of tissue architecture. I believe that a detailed understanding of the mechanism by which cell architecture is disrupted during cancer will provide new insights into our understanding precancerous lesions that will not only identify a new class of drug targets, but also identify a new class of biomarkers that can be used to predict prognosis of premalignant lesions.

All malignant breast cancers originate from epithelial cells. In normal breast tissue, epithelial cells line the ductal structures and are involved in secretion of milk. These cells have a characteristic architecture referred to as apical-basal polarity, where the apical surface faces the lumen and the basal-lateral surface is in touch with the surrounding microenvironment. The molecular mechanisms by which the epithelial cells establish and maintain polarity is an active area of investigation. There are several proteins and protein complexes that function as a highly interactive network during establishment and maintenance of epithelial cell polarity. One such complex, the Par6/aPKC/Cdc42, regulates polarization processes during epithelial morphogenesis, astrocyte migration, and axon specification. We and other investigators have demonstrated that this complex is also required for disruption of apical-basal polarity during oncogene ErbB2-induced transformation and transforming growth factor-β (TGF-β)-induced invasion of mammary epithelial cells.

Our recent results show that expression of Par6 in mammary epithelial cells induces epidermal growth factor (EGF)-independent cell proliferation and development of hyperplastic three-dimensional acini without affecting apical-basal polarity. This was dependent on the ability of Par6 to interact aPKC and Cdc42 but not Lgl and Par3 and its ability to promote sustained activation of MEK/Erk signaling. Interestingly, Par6 is overexpressed in estrogen-receptor-positive (ERtve) primary human breast cancers and in ERtve precancerous breast lesions. Either Par6β itself or the pathways it regulates may be excellent candidates for predictive biomarkers or drug targets for premalignant lesions.

Loss-of-function mutations in polarity genes, such as *Scribble*, induce neoplastic overproliferation of epithelial cells in *Drosophila*, suggesting that polarity genes function as tumor suppressors by regulating cell proliferation. The role polarity genes, including *Scribble*, have during tumorigenesis in mammals is poorly understood. We have discovered that down-regulation of *Scribble* promotes Myc-induced transformation of mammary epithelial cells in culture and in mouse models of breast cancer by blocking Myc-induced cell death with no detectable effect on proliferation. Surprisingly, *Scribble* is required for Myc to activate the Rac-JNK (c-Jun amino-terminal kinase) pathway and trigger apoptosis. Inhibition of Rac or JNK activity phenocopied *Scribble* loss and blocked Myc-induced apoptosis, indicating that the *Scribble*-Rac-JNK axis of signaling is a novel regulator of Myc-induced transformation of mammary epithelial cells. In addition, loss of *Scribble* blocks luminal apoptosis during acinar morphogenesis of breast epithelial cells in three-dimensional culture and promotes development of dysplastic mammary ductal

outgrowth in vivo. Our results demonstrate that *Scribble* regulates cell death pathways during normal morphogenesis and Myc-induced transformation of mammary epithelial cells. Thus, in mammals, *Scribble* functions as a tumor suppressor by regulating cell death.

NOVEL TARGETS FOR ERBB2-POSITIVE BREAST CANCERS

ErbB2 belongs to the EGF-receptor (EGF-R) family of receptor tyrosine kinases. Amplification of *erbB2* occurs in about 25% of breast cancers and is correlated with a poor clinical outcome. Although ErbB2 is not activated by direct binding of soluble ligands, it is activated by ligand-induced formation of heterodimers with other EGF-R family members, namely, EGF-R/ErbB1, ErbB3, and ErbB4. Overexpression of ErbB2 can also result in ligand-independent receptor homodimerization and activation. Overexpression of *neu* (the mouse homolog of human *erbB2*) under the control of mouse mammary tumor virus (MMTV) long terminal repeat (LTR) results in mammary tumorigenesis, suggesting that *erbB2* can initiate tumorigenesis in vivo.

ErbB2 is a drug target for human breast cancer. A humanized monoclonal antibody against the extracellular domain of ErbB2, Herceptin, is used to treat patients expressing high levels of ErbB2 in combination with chemotherapy. In patients who respond to the treatment, Herceptin can delay mortality anywhere between 9 months to 3 years. However, almost 50% of the patients with tumors that contain erbB2 amplification do not respond to Herceptin and those who do respond develop resistance to the drug, highlighting the need for better options to treat patients with ErbB2-positive cancers. A better understanding of the ErbB2 signaling pathway can identify novel targets for combination therapy that will significantly aid in our ability to treat patients with ErbB2-amplified breast cancers.

Members of the ErbB2 signaling pathway can also be coamplified with ErbB2 in breast cancer. For example, Grb7, a Src homology domain (SH2) containing an adaptor molecule that associates with ErbB2 and amplifies signaling by ErbB2, is a component of the ErbB2 amplicon. In addition to the *erbB2* amplicon, *erbB2*-amplified tumors also possess several gains and losses located elsewhere in the genome. Whether these genomic alterations contain novel members of the ErbB2 signaling pathway that are involved in initiation, progression, and maintenance of *erbB2*-amplified breast tumors is poorly understood.

We have discovered that Brk, a cytoplasmic tyrosine kinase, is coamplified and coexpressed with ErbB2 in human breast cancers. ErbB2 interacts with Brk and increases its intrinsic kinase activity. Expression of Brk enhances the ErbB2-induced activation of Ras/MAPK (mitogen-activated protein kinase) signaling and cyclin E/cdk2 activity to induce cell proliferation of mammary three-dimensional acini in culture. To investigate whether ErbB2 and Brk cooperate during mammary tumorigenesis in a mouse model, we developed a novel mammary fat pad transplantation system to generate transgenic mammary tissue. This approach involves the use of pluripotent mammary epithelial cells engineered ex vivo that can be transplanted into epithelium-free mammary fat pads.

Using this model, we show that expression of Brk shortened the latency of ErbB2-induced tumors by promoting cell proliferation with no effect on protection from apoptosis. Furthermore, overexpression of Brk confers resistance to the ability of Lapatinib, an ErbB2 kinase inhibitor, to inhibit ErbB2-induced proliferation. Thus, we identified Brk as a novel drug target for ErbB2-positive cancers. In addition, this study serves as a proof-of-principle for conducting a large-scale investigation to identify novel targets for ErbB2-positive breast cancers. Thus, we have made key advances both in finding insights into the role polarity proteins have during early stages of breast cancer and in finding a new target for ErbB2-positive breast cancers.

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CELL SIGNALING IN HIV PATHOGENESIS

J. Skowronski M. Gierszewska M. Kwofie
K. Hrecka S. Srivastava

Our interest lies in understanding the molecular mechanisms that underlie the pathogenesis of AIDS and, in particular, understanding the functional consequences of interactions between viral proteins and the regulatory machineries in the infected cells. The main focus of our research is to understand the functions of Nef, Vpr, and Vpx accessory virulence factors of human and simian immunodeficiency viruses (HIV and SIV). We have directed a major effort toward the identification of mechanisms and downstream effectors that mediate the effects of Nef and Vpr in the infected cells, and our experiments have been concentrated in two main areas.

One major focus has been on the isolation and identification of cellular proteins that mediate the effects of Nef on signal transduction and endocytic machineries. This has led to the purification and microsequencing of several cellular factors that associate with Nef in T lymphocytes. Importantly, one of Nef targets is a critical molecular switch that regulates Rac GTPases downstream from chemokine- and antigen-initiated signaling pathways. This interaction enables Nef to influence multiple aspects of T-cell function and thus provides an important mechanism by which Nef impacts pathogenesis by primate lentiviruses.

The other major focus has been on identification of downstream effectors of lentiviral Vpr and Vpx accessory proteins. These experiments have led to the description of several novel cellular proteins that tightly associate with these factors in hematopoietic cells. Among them are E3 ubiquitin ligase complexes that regulate cell cycle progression and the repair of damaged DNA. The observation that Vpr tightly associates with E3 ubiquitin ligase components is interesting because HIV replication is known to be restricted by protein ubiquitination. Remarkably, we found that both the Vpr and Vpx proteins target a novel substrate adaptor for Cullin 4 and function through their associated Cullin-4 E3 ubiquitin ligase complexes. Here, we describe in more detail our studies of lentiviral Vpr.

FUNCTIONS OF VPR AND VPX LENTIVIRAL ACCESSORY FACTORS

Vpr and Vpx are multifunctional accessory proteins of simian and/or human immunodeficiency viruses (HIV-1, HIV-2, and SIV). One function of HIV-1 Vpr is to mediate translocation of viral reverse transcription complexes into the nucleus in nondividing cells, such as terminally differentiated macrophages. The other is to perturb the cell cycle progression of the infected cell. Both functions are thought to be important for facilitating the HIV-1 life cycle in the infected host. Unlike HIV-1, which possesses only the *vpr* gene, HIV-2 and SIVmac specify both the *vpr* and a closely related *vpx* gene. Interestingly, the Vpr proteins encoded by SIV and HIV-2 block cell cycle progression in the G_2/M phase similar to HIV-1 Vpr but do not have the ability to promote nuclear transport of the preintegration complexes in nondividing cells. This latter function is thought to be executed by Vpx accessory factors encoded by these HIV-2/SIV strains. Thus, two separable functions of the HIV-1 Vpr protein are encoded by distinct polypeptides in HIV-2/SIV. We thought that comparative studies of host-cell proteins that associate with Vpr/Vpx would provide a interesting system in which to analyze the molecular mechanisms mediating the cell cycle and nuclear transport effects of these accessory proteins.

VPR AND VPX ASSOCIATE WITH CATALYTIC AND REGULATORY COMPONENTS OF CULLIN-4-BASED E3 UBIQUITIN LIGASE COMPLEXES

We used a combination of biochemical and proteomic approaches to identify cellular proteins associated with human and simian Vpr/Vpx proteins. Briefly, Vpr (or Vpx) and its associated proteins were purified from cells by two sequential immunoprecipitations and analyzed by multidimensional protein identification technology (MudPIT). MudPIT is a combination of chromatographic and mass spectrometric procedures that allow unbiased and sensitive identification of proteins in complex mixtures. These experiments were carried out in collaboration with Drs. Michael Washburn, Laurence Florens, and Selene Swanson of Stowers Institute for Medical Research (Kansas City, Kansas).

The MudPIT analysis identified three relatively abundant polypeptides—DDB1, DDA1, and VprBP/DCAF1—that were specifically and abundantly associated with both HIV-1 and SIVmac Vpr proteins, as well as with SIVmac Vpx. DDB1 is an obligatory subunit of

all known E3 ligase complexes assembled on a Cullin-4 (Cul4) scaffold. VprBP/DCAF1, a known HIV-1 Vpr-binding protein recently shown to bind DDB1, is thought to function as one of many substrate receptors that recruit cellular proteins for ubiquitination by the E3 enzyme. DDA1 was also shown to bind DDB and has been implicated for regulation of E3 ligase catalytic activity. Thus, our findings tentatively linked Vpr to Cul4 RING E3 (Cul4 E3) ubiquitin ligases. These enzymes are known to regulate DNA repair and replication through ubiquitination of key components of these processes.

Our subsequent biochemical studies revealed that DDA1, DDB1, and VprBP/DCAF1 form a ternary complex, which is then targeted by Vpr, and that this complex links Vpr and Vpx proteins to the catalytic core of the ubiquitin ligase organized on the Cul4 scaffold. The notion that Vpr proteins target a Cul4-based E3 ubiquitin ligase is interesting because previous evidence indicated that replication of primate lentiviruses is restricted to some extent by protein ubiquitination. These findings raised the possibility that Vpr/Vpx promote lentivirus replication by modulating protein ubiquitination in infected cells.

VPRBP REGULATES CELL CYCLE

As aforementioned, Cul4 E3 ubiquitin ligases are thought to control DNA replication and progression of the cell cycle. We hypothesized that Vpr usurps VprBP/DCAF1 and its associated Cul4 E3 to perturb these processes. To address this possibility, we characterized the normal function of VprBP using RNA interference and functional assays. Strikingly, cell cycle analyses revealed that the majority of VprBP depleted cells in the G_1 and G_2 phases. These findings indicated that VprBP/DCAF1 is essential for progression of the cell cycle.

Cell cycle arrests at the G_1/S border and in G_2 frequently reflect activation of DNA damage checkpoints. To assess whether VprBP/DCAF1 depletion activates checkpoint responses, we next analyzed expression of key checkpoint proteins and cell cycle regulators in VprBP/DCAF1-depleted cells. We found that the steady state of several proteins involved in the cellular response to DNA damage, such as Ser-139-phosphorylated histone H2A.X variant (γ-H2A.X), as well as the p53 and p21 cyclin-dependent kinase inhibitor, which mediates the p53-dependent DNA-damage checkpoint (35), were all increased in VprBP-depleted cells. This indicated that VprBP/DCAF1-depleted cells arrest at DNA-damage checkpoints. Furthermore, these observations implicate a role for VprBP/DCAF1 in DNA replication and/or cellular response to damaged DNA.

HIV-1 VPR ARRESTS CELL CYCLE BY INTERACTING WITH THE CUL4-DDB1[VPRBP] E3 COMPLEX

One conserved function of Vpr proteins is their ability to arrest cells in the G_2 phase of the cell cycle at the DNA-damage checkpoint. Our finding that VprBP/DCAF1 regulates the cell cycle and interacts with checkpoint responses suggested that Vpr could arrest the cell cycle through its interaction with VprBP/DCAF1 and its associated Cul4 E3 ubiquitin ligase. To address this possibility, a panel of HIV-1 Vpr alleles were tested for their abilities to arrest cells in G_2 and to bind VprBP and its associated Cul4 E3. Remarkably, we found perfect correlation between the ability of Vpr to associate with the VprBP and its associated Cul4 and to arrest cells in the G_2 phase of the cell cycle. This evidence strongly supports the possibility that Vpr manipulates the cell cycle by modulating the ubiquitin proteasome system through its interaction with VprBP/DCAF1-associated Cul4 ubiquitin ligase. Current studies address how Vpr alters protein ubiquitination and how this impacts the lentivirus life cycle.

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

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According to the American Cancer Society, non-small-cell lung carcinoma (NSCLC) is the leading cause of death from cancer in the United States. Most patients with advanced NSCLC have a median survival of 4–5 months after diagnosis, and a 1-year survival of less than 10%. The use of conventional chemotherapies for NSCLC usually results in a very modest survival increase, but at the cost of significant toxicity.

The development of "smart" drugs—targeting specific oncogenes that tumors depend on for survival—has proven to be an effective therapeutic strategy in our attempt to combat cancer and has therefore generated much excitement in recent years. Such therapies are associated with limited side effects and thus, wider therapeutic windows of intervention. Successful examples of targeted therapies include monoclonal antibodies against cell surface proteins, i.e., Herceptin® (trastuzamab) and Erbitux® (cetuximab); small-molecule ATP antagonists targeting tyrosine kinase activity, i.e., Gleevec® (imatinib), Iressa® (gefitinib), and Tarceva® (erlotinib); and selective transcription factor inhibitors, i.e., Tamoxifen.

Phase II and III clinical trials of small-molecule inhibitors targeting epithelial growth factor receptor (EGFR) tyrosine kinase activity, namely, gefitinib and erlotinib, have indicated that a small subset of NSCLC patients benefit substantially from EGFR inhibitor therapy. Recently, we provided evidence showing that the presence of specific EGFR somatic mutations correlates highly with the positive dramatic response observed in this subset of NSCLC patients. These mutations affect four exons spanning the kinase domain of the receptor. We demonstrated that the presence of these mutations substantially alters the biochemical properties of EGFR.

The studies conducted in our laboratory are aimed at understanding how and why cancer cells become dependent on mutant EGFR for their survival and how, in instances of acquired resistance, these same cells become refractory to EGFR-selective inhibition.

Functional Analysis of Mutant EGFR Signaling Pathways

T. Linsted, C. Biot, A. Ratner, K. Melgar

One of the main goals of our studies is to understand why inhibition of EGFR results in cancer cell death (oncogene addiction). Several theories have been proposed to provide a molecular explanation to cancer cell dependency on the activity of certain oncogenes. It has been hypothesized that the presence of selective oncogenes (e.g., mutant EGFR) can lead to a rearrangement of preexisting cellular signaling networks resulting in an increased vulnerability to apoptotic stimuli. More recently, our colleague Dr. Jeff Settleman at the Massachusetts General Hospital proposed an alternative theory termed "oncogenic shock." On the basis of this theory, oncogenes activate proapoptotic and antiapoptotic signaling pathways characterized by different kinetic of inactivation. As a consequence of oncogene inactivation, long-lived proapoptotic pathways will not be effectively balanced by prosurvival short-lived signaling pathways. This imbalance in signaling supposedly results in cancer cell death.

We and other investigators have shown that mutant EGFRs are characterized by an augmented kinase activity and an increased activation of AKT and STAT prosurvival pathways. We are currently studying the molecular mechanism underlying these qualitative signaling differences. In addition, we are investigating whether and how these same signaling pathways play a part in determining cell addiction to mutant EGFR.

Identification of New Determinants of Erlotinib and Gefitinib Sensitivity

S. Fenoglio

Retrospective studies have showed a high degree of correlation between the presence of EGFR somatic muta-

tions and responses to erlotinib and gefitinib treatment. Yet not all patients harboring mutations within the EGFR locus will respond to gefitinb and erlotinib treatment, although other patients, whose tumors express the wild-type EGFR allele only, have been reported to show a partial response to EGFR-selective inhibition.

More recently, clinical and experimental data have also indicated that the lengths of the response to gefitinib and erlotinib treatment are highly variable, occasionally lasting for more than 4 years; however, in most cases, relapses are observed as early as 6 months. The presence of secondary mutations within the EGFR locus and amplification of the c-*met* gene have been specifically suggested to be important mechanisms of acquired resistance.

We are interested in uncovering new determinants of the cellular response to EGFR inhibition with the goal of developing new therapeutic options for NSCLC treatment.

To this aim, we developed an in vitro cell-based system that leads to the identification of SOCS2 as a potential player in gefitinib and erlotinib primary and acquired resistance. We are currently investigating the molecular mechanism underlying the increased resistance observed in cells overexpressing SOCS2.

Interestingly, we found that SOCS2 expression is regulated in NSCLC by estrogen receptor activity and that, in the context of NSCLC, estrogen antagonizes the oncogenic potential of mutant EGFR by regulating the expression of SOCS2. On the basis of these data, we are investigating whether low levels of estrogen receptor activity and SOCS2 are required for the establishment of tumors driven by mutant EGFR. These findings could provide new therapeutic options and could potentially explain the observation that in postmenopausal women, in which levels of estrogen are particularly low, lung tumors containing mutant EGFR are more common.

Identification of New NSCLC Therapeutic Targets

H. Archibald, A. Torres

On the basis of the successes of gefitinib and erlotinib, our laboratory is also interested in uncovering new oncogenes that, similar to EGFR, can be used as therapeutic targets for the treatment of NSCLC. To this aim, we are utilizing functional genetic screens that will allow us to uncover signaling pathways and genes to which NSCLC cells rely on for their survival. The experience with gefitinib and erlotinib taught us that, in some cases, molecular-targeted therapies will work in only a small subset of patients. The low frequency of genetically defined responsive patient subsets thus calls for consideration of a broader sampling to achieve a representation of genetic diversity. For example, to detect a response similar to the one observed in the case of gefitinib and erlotinib treatment in NSCLC patients (i.e., 10%), we can estimate that a cell-based screen would require a minimum of 50 cell lines.

On the basis of this consideration, we collected in the past year more than 80 NSCLC-derived cell lines. By using a compound-based screen, we were able to classify the cell lines of our in-house collection in several clusters characterized by different sensitivities to a large pool of compounds targeting signaling pathways known to be deregulated in tumors. As a result, we identified a subset of cell lines that display high sensitivity to PDGFR-selective inhibitors. We are currently investigating the molecular basis of their selective sensitivity and, although the benefit of a PDGFR-based therapy in the treatment of NSCLC has yet to be elucidated, in collaboration with Dr. Scott Powers's group here at CSHL, we are attempting to identify genetic markers that can be used in the clinic for the upfront selection of tumors that will respond to PDGFR-selective inhibition.

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PROTEIN TYROSINE PHOSPHATASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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F. Chaudhary L. Li D. Vasudevan
A. Haque G. Lin X.C. Zhang

The phosphorylation of tyrosyl residues in proteins is a key component of the regulation of signaling pathways that control many fundamental physiological processes, including cell proliferation and differentiation. Protein phosphorylation is a reversible, dynamic process in which the net level of phosphate observed in a target substrate reflects the coordinated activity of the kinases that phosphorylate it and the protein phosphatases that catalyze the dephosphorylation reaction. We study the family of protein tyrosine phosphatases (PTPs), which, like the kinases, comprise both transmembrane receptor-linked forms, as well as nontransmembrane cytoplasmic species and represent a major family of signaling enzymes.

Overall, the objective of the lab is to develop tools for analysis of PTP regulation and function and integrate them with state-of-the-art cell and animal models to define critical tyrosine-phosphorylation-dependent signaling events in human disease. It is anticipated that these studies will lead to identification of novel therapeutic targets and biomarkers at two levels. By elucidating the signaling function of PTPs in disease models, we will reveal examples in which the PTPs themselves, or regulators of PTP function, are targets. Furthermore, we will use the PTPs as probes to define critical signaling pathways in human disease from which novel targets will be identified.

There was significant turnover in our lab during the last year. Deirdre Buckley went back home to Ireland to take up the position of Senior Research Scientist at the Biosciences Institute, University College Cork. Naira Gorovits became a Scientific Analyst in the law firm of Jacob, Medinger, and Finnegan LLP. Antonella Piccini returned to Italy as a Research Associate at the NATO Institute in Trieste. Shanta Hinton joined the faculty of Hampton University, Virginia, as an Assistant Professor in the Department of Biological Sciences.

Seung-Jun Kim came to the lab as a Visiting Scientist from the Korea Research Institute of Bioscience & Biotechnology at the University of Science & Technology in Korea. Ulla Schwertassek joined us as a postdoctoral fellow, having completed her Ph.D. in the lab of Dr. Tobias Dick at the German Cancer Research Center (DKFZ) in Heidelberg. In addition, two graduate students, Fauzia Chaudhary and Deepika Vasudevan, from the Molecular and Cellular Biology Program at Stony Brook, began their Ph.D. studies. Finally, Xiaoqun Catherine Zhang joined the group as a Research Associate.

FUNCTIONAL ANALYSIS OF THE "PTPOME"

We have now identified all of the PTP genes in the human genome (there are ~100) and more recently have extended our genomic sequence analysis to other organisms. To date, various studies from many labs have pointed to the functional importance of the PTPs in the control of cell signaling. It is now apparent that the PTPs have the capacity to function both positively and negatively in the regulation of signal transduction. Furthermore, the PTPs have the potential to display exquisite substrate, and functional, specificity in vivo. Nevertheless, the majority of the members of the PTP superfamily have yet to be characterized. Hence, the PTPs remain a largely untapped resource that can be exploited to reveal new insights into the regulation of signal transduction. Having defined the composition of the PTP superfamily, the overall goal of this aspect of the work in the lab is to develop the tools and reagents for a functional analysis of the "PTPome," i.e., to analyze the function of members of the PTP superfamily, under normal and pathophysiological conditions, from the perspective of the family as a whole.

During the past year, we have completed construction of a library of short hairpin RNAs (shRNAs) that targets each of the PTPs (five shRNAs for each PTP) and therefore will allow us to interrogate systematically the function of the members of this family of signaling enzymes by RNA interference (RNAi) in various disease models. In addition, we have been assembling a collection of antibodies to each of the PTPs, to facilitate analysis of protein suppression by RNAi, and a collection of expression plasmids for each of the PTPs, to facilitate follow-up studies on candidates identified in RNAi screens.

Our first application of this library is being con-

ducted in collaboration with Senthil Muthuswamy, who is an expert in cell models of breast cancer, here at CSHL. We have begun by using his three-dimensional (3D) cell culture system, which recapitulates several aspects of mammary gland architecture in vivo and has been used to model the biological activities of cancer genes, including the oncoprotein tyrosine kinase HER2 (ErbB2), which is overexpressed in about 25% of breast tumors. In 3D culture, mammary epithelial cells undergo a morphogenetic program that culminates in the formation of multicellular structures that are reminiscent of acini in vivo. We are investigating the effects of attenuating PTP expression on the ability of MCF10A mammary epithelial cells to undergo differentiation into acini and on the ability of HER2 to interfere with that process.

Thus far, we have observed that suppression of a receptor PTP, PTPRO, led to an increase in acini size, which was associated with an extended period of proliferation, without effect on apoptosis. In addition, we observed that suppression of PTPRO enhanced the proliferation and formation of multi-acinar structures induced by HER2, suggesting a previously unknown function for PTPRO in down-regulating signaling pathways triggered by HER2. In parallel, we tested the effects of suppressing DEP-1, a receptor PTP that was first identified in this lab and has been suggested to have a tumor suppressor function in colon. In contrast to PTPRO, suppression of DEP-1 alone was sufficient to induce a multi-acinar phenotype in MCF10A cells in 3D culture. These experiments suggest that different PTPs exert distinct effects in the control of acinar development, presumably through differences in substrate specificity and through regulation of distinct signaling pathways. Our studies to date have now established the methodology for a systematic screen for the effects of PTP superfamily members on mammary epithelial cell differentiation and we are preparing to conduct such a screen.

In the interim, we have proceeded with a distinct screen to define the function of PTPs in the regulation of HER2-induced cell motility. In this case, we are using MCF10A cells that express an inducible HER2 protein and testing for the effects of PTP suppression on cell migration in Transwell chambers, in the presence or absence of a small-molecule dimerizer that activates the kinase. In a proof-of-concept assay, we tested the effects of suppressing PTP1B expression. Recent studies from the Tremblay (McGill University, Montreal) and Neel (Beth Israel Deaconess Medical Center, Harvard Medical School) labs have shown that when mice expressing activated alleles of HER2 were crossed with *ptp1B*-knockout mice, tumor development was delayed and the incidence of lung metastases was decreased. It has also been shown that PTP1B is overexpressed in HER2-transformed cells. Thus, PTP1B, which has a well-established role in down-regulating insulin and leptin signaling, appears also to have a *positive* role in regulating signaling events associated with breast tumorigenesis. We noted that suppression of PTP1B attenuated HER2-induced migration of MCF10A cells consistent with such a positive signaling function. Interestingly, suppression of the closely related enzyme TCPTP was without effect. We are now examining the substrate specificity of PTP1B in this context to define its mechanism of action. In addition, we have almost completed our systematic analysis of the whole enzyme family in this screen and have identified five PTPs for which suppression enhanced HER2-dependent migration and one PTP, suppression of which attenuated migration. In collaboration with Jim Hicks and Mike Wigler, here at CSHL, we are also examining changes in expression, in DNA methylation or mutations in these PTPs in breast cancer compared to normal tissue. Currently, we are defining optimal conditions for further analysis of the substrate specificity and mechanism of action of the PTPs identified in this screen. This approach shows great potential to shed new light on the roles of PTPs in the etiology of breast cancer.

REGULATION OF PTP FUNCTION BY REVERSIBLE OXIDATION

The signature motif of the PTP family, [I/V]HCxxGxxR[S/T], contains an invariant Cys residue, which, due to the unique environment of the active site, is characterized by an extremely low pKα. This promotes the function of this Cys residue as a nucleophile in catalysis, but it renders it highly susceptible to oxidation, with concomitant abrogation of nucleophilic function and inhibition of PTP activity. Work from several labs, including this one, has now established that multiple PTPs are transiently oxidized and inactivated in response to a wide array of cellular stimuli, which represents a novel tier of control of tyrosine-phosphorylation-dependent signaling. Hence, the ability to detect reversible oxidation of PTPs in vivo is critical to understanding the complex biological role of reactive oxygen species (ROS) in the control of cellular signaling.

During the last year, we have completed development of a new assay to measure reversible PTP oxidation in cells. A manuscript describing this work is currently under review. The assay utilizes the unique chemistry of the invariant catalytic Cys residue in labeling the active site of reversibly oxidized PTPs with biotinylated small

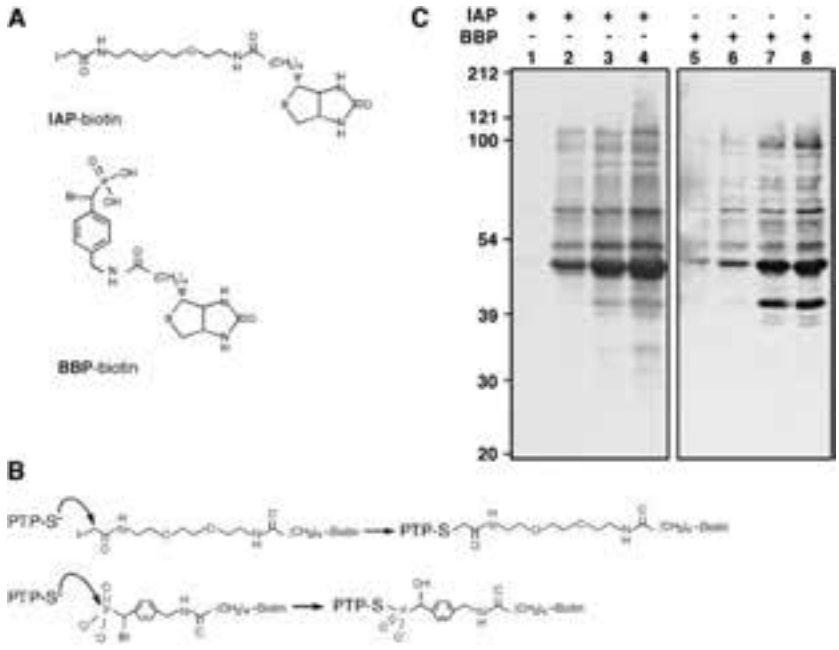

FIGURE 1 Comparison of IAP- and BBP-based probes for detection of reversibly oxidized PTPs in PDGF-BB-transformed angiomyolipoma cells. Angiomyolipomas are benign tumors of the kidney that express functional PDGF-R. This experiment was carried out in collaboration with Jack Arbiser (Emory University) using cells that were derived in his lab from an angiomyolipoma, by immortilization following sequential introduction of SV40 large T and telomerase (SV7tert). These cells express active PDGF-Rβ but do not form tumors in nude mice. However, upon constitutive retroviral expression of PDGF-BB, the cells became tumorigenic and produced high levels of ROS. In this experiment we compared PTP oxidation in immortalized but untransformed SV7tert cells, PDGF-BB-transformed cells, and two tumor-xenograft-derived PDGF-transformed cell populations (Tumor 1 and Tumor 2 cells). Cells were lysed under anaerobic conditions in the presence of iodoacetic acid (IAA), an alkylating agent that modifies covalently the thiolate anion of the active-site Cys in those PTPs that remained in a reduced state. In contrast, any PTPs that were oxidized by the localized stimulus-induced production of ROS were protected from this irreversible alkylation. Lysates were then subjected to buffer exchange on a size-exclusion chromatography column. In this key step, IAA was removed and the oxidized PTPs were reduced back to the active state, in which they may react with biotinylated active-site-directed compounds. Biotinylated proteins were purified on streptavidin–Sepharose beads, resolved by SDS-PAGE, and visualized using streptavidin-HRP. (*A*) Structures of biotinylated IAP and BBP probes; (*B*) mechanisms of PTP labeling by the IAP and BBP probes. (*C*) Serum-deprived (16 hours) angiomyolipoma cell lines (*1,5:* SV7tert; *2,6:* SV7tert-PDGF; *3,7:* SV7tertPDGF-Tumor1; *4,8:* SV7tertPDGF-Tumor2) were subjected to the cysteinyl-labeling assay using either biotinylated IAP (*1–4*) or BBP probes (*5–8*).

molecules under mildly acidic conditions (Fig. 1). The two biotinylated compounds used were a sulfydryl-reactive iodoacetylpolyethylene oxide (IAP) probe and an α-bromobenzylphosphonate (BBP) activity-based probe (in collaboration with Zhong-Yin Zhang, Indiana University). Those PTPs that were oxidized in the original sample can then be identified following purification by streptavidin pull-down and visualization with streptavidin-HRP (horseradish peroxidase). We have applied this cysteinyl-labeling assay to the study of platelet-derived growth factor (PDGF) receptor signaling in an angiomyolipoma cell model. Interestingly, there were close similarities in the pattern of reactive proteins that were observed in both IAP- and BBP-labeling assays, suggesting that this strategy leads to specific detection of oxidized PTPs (Fig. 1). The composition of the PTP superfamily has been defined, and thus the apparent molecular weight of the reactive proteins can be used to predict identity, which can subsequently be tested by immunoblotting for candidate PTPs. We have detected PDGF-induced reversible oxidation of members of both classes of classical PTPs (receptor-like and nontrans-

membrane PTPs) and of two different classes of dual specificity phosphatases (DSPs) (VH1-like and PTEN-like), which demonstrates the broader sensitivity of this improved assay in comparison to preexisting techniques, allowing us to detect representatives of all the major subgroups of the PTP superfamily. Currently, in collaboration with various labs, we are applying this cysteinyl-labeling enrichment strategy to study reversible PTP oxidation in a variety of signaling pathways, which will help delineate new signaling cascades and improve our understanding of the complex biology of ROS signaling in vivo.

During the past year, results from our collaboration with T.C. Meng, Kay-Hooi Khoo, and their colleagues at the Academia Sinica in Taiwan have been published. We have used mass spectrometry to characterize the oxidation status of each Cys residue in PTP1B that had been isolated by immunoprecipitation from HepG2 and A431 human cancer cells, which produce high levels of ROS constitutively. We observed that up to 50% of PTP1B was reversibly oxidized, and, due to the presence of the high ROS levels produced constitutively in these cells, we observed that up to 40% of PTP1B was irreversibly oxidized. In addition, our data show that the oxidation of PTP1B is specific to the active site Cys, with the other Cys residues in the protein remaining in a reduced state. Our data also suggest that the high level of intrinsic ROS may contribute to the transformed phenotype of HepG2 and A431 cells via constitutive inactivation of cellular PTPs. Furthermore, it may be possible now to apply this approach to determining the stoichiometry of oxidation of specific PTPs in various signaling contexts.

FUNCTIONAL ANALYSIS OF JSP1

Previously, we identified JSP1 (JNK stimulatory phosphatase 1) as a DSP that has the capacity to activate the JNK mitogen-activated protein kinases (MAPKs) specifically, exerting its effects upstream of the MAP2K enzyme MKK-4 in the JNK signaling cascade. This illustrates a new potential tier of control of the JNK signaling pathway and a novel aspect of the role of protein phosphatases in the control of MAPK signaling, raising the possibility that JSP1 may offer a new perspective on the study of various disorders associated with aberrant JNK signaling. We have been characterizing mice that contain a targeted deletion at exon 3 of the *jsp1* gene, resulting in the production of a truncated, inactive JSP1 protein of approximately 20 amino acids. These mice reproduce at normal Mendelian ratios, display no obvious physical abnormalities, and have a life expectancy similar to that of their wild-type controls. We are characterizing two aspects of their phenotype, one of which indicates that JSP1 may have a role in the neurodegenerative processes that lead to Parkinson's disease, whereas the other implicates JSP1 in the regulation of innate and adaptive immunity.

Regulation of JNK signaling by JSP1 in a mouse model of Parkinson's disease. Parkinson's disease (PD), one of the most prevalent neurodegenerative diseases affecting aging individuals, is characterized by disabling motor defects arising from a progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). Less than 10% of PD cases have been linked to genetic factors and therefore the disease is thought to be primarily sporadic in nature with unknown etiology. Animal models of PD primarily involve the use of neurotoxins, the most common being MPTP (1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine), administration of which leads to dopaminergic neuronal cell death and a Parkinsonian phenotype. Apoptosis is the key cell death mechanism in both PD and in MPTP-induced lesions, with the JNK signal transduction pathway having a major role in this process. There is significant interest in targeting the JNK pathway for PD therapeutics; however, global inhibition of JNK activity would be likely to have detrimental systemic affects, and therefore it is important to identify key regulators of JNK signaling in the context of neuronal cell death in PD.

To investigate the possibility that JSP1 may regulate JNK signaling in the context of neuronal stress, we have been examining the effects of MPTP on c-JUN phosphorylation in the SNpc of wild-type and *jsp1*$^{-/-}$ mice. These studies are being performed with Serge Przedborski and his colleagues at Columbia University. Using confocal fluorescence microscopy, we had observed significant phosphorylation of c-JUN in the dopaminergic neurons of wild-type mice at 6 hours post-MPTP treatment, but this was dramatically lower in *jsp1*$^{-/-}$ mice. This indicates that JSP1 may act positively to regulate MPTP-mediated JNK signaling in dopaminergic neurons. Our efforts during the last year have been focused on quantitation of these effects using laser scanning cytometry (iCys®) and we have been working in collaboration with Ed Luther at Compucyte to overcome a variety of technical challenges. In particular, we had to distinguish MPTP-induced JNK from the high basal level of activity in brain tissue. Furthermore, we have encountered a high-background antibody staining, which is a consequence of the fixation conditions we have to use. Nevertheless, we feel we have now opti-

mized conditions, and our data indicate that in the absence of *jsp1*, the activation of JNK signaling is delayed. The next phase of this project will address the substrates of the phosphatase that underlie these effects.

Modulation of innate and adaptive immunity by JSP1. Our recent analysis of JSP1 knockout mice has revealed that aged *jsp1*$^{-/-}$ animals (>1 year) develop moderate splenomegaly, and this is associated with lymphoid hyperplasia and an expansion of memory T cells. At an early age (1-month-old mice), *jsp1*$^{-/-}$ naïve CD4^{+} T cells display a reduced proliferative capacity compared to wild type, as measured by thymidine incorporation and interleukin-2 (IL-2) production. However, at 4 months, *jsp1*$^{-/-}$ naïve CD4^{+} T cells display a moderately increased proliferative capacity compared to wild type. This apparent disparity can be explained by the observation that wild-type CD4^{+} T cells undergo a dramatic decline in proliferative capacity as they age from 1 to 4 months. In contrast, although *jsp1*$^{-/-}$ CD4^{+} T cells from young mice do not display as robust a response to T-cell activation as their wild-type counterparts, they display no significant age-dependent decline in proliferation. These data, and the observation that aged *jsp1*$^{-/-}$ mice possess an increased memory T-cell pool, indicate that JSP1 may have important roles in aging of the adaptive immune system. Our most recent data suggest that ablation of JSP1 disrupts NFAT (nuclear factor of activated T cell) signaling, and our current efforts are focused on defining the mechanism for these effects.

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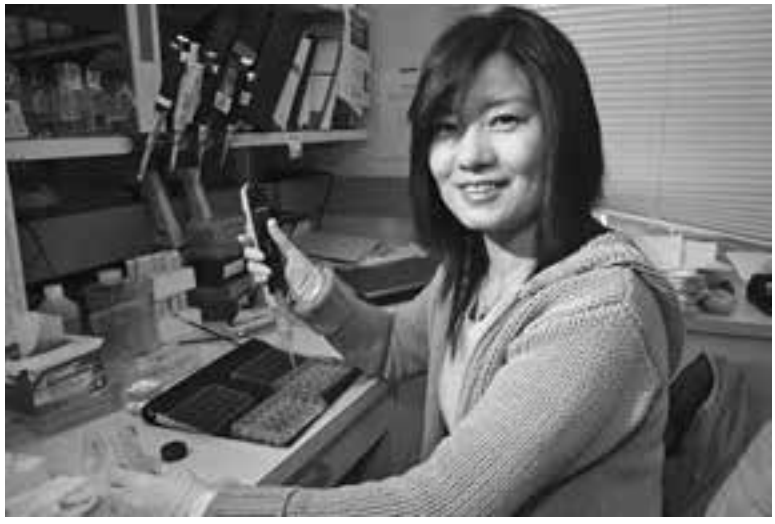

Li Li

EMERGING PRINCIPLES OF TUMOR SUPPRESSION

L. Trotman M. Chen
H. Cho
J. Williams

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 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 an early cell-intrinsic human antitumoral defense mechanism. However, we have found that cells fail to respond similarly to more subtle deregulation of Pten protein as exemplified by its incomplete loss and that this leads to cancer by a process that we term "senescence bypass." This finding combined with the senescence response suggests that tumors should favor retention of some PTEN. Indeed, many human cancers present with incomplete *PTEN* loss, entirely consistent with these findings and establishing *PTEN* as a haploinsufficient tumor suppressor in such tissues.

Our studies of senescence bypass mechanisms have so far revealed two molecular scenarios. In one, tissues will spontaneously reduce *Pten* levels via the ubiquitin pathway just enough to cross the threshold for abnormal proliferation. In the second scenario, partial inactivation of *Pten* and a select cooperating tumor suppressor (e.g., the nuclear body-forming protein Pml or the cell cycle inhibitor p27) will suffice to produce senescence-free cancers that form only in this specific combination. Importantly, analysis of human tissues has confirmed the relevance of these two scenarios in prostate and colon cancer patients to name a few. Thus, our genetic analysis of tumor progression in the mouse is allowing us to develop and test novel conceptual and mechanistic frameworks for cancer initiation and progression.

TUMOR SUPPRESSOR COOPERATION IN CANCER

Through our previous studies, we have learned that not only quantitative regulation, but also qualitative regulation of Akt kinase controls *Pten* mutant tumorigenesis and its progression via a senescence defeat or senescence bypass pathway. The qualititve change in active Akt kinase localization from the plasma membrane to the nucleus is a direct consequence of loss of 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 Akt partitioning via control of phosphatase activity 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. However, it is unclear (1) whether PHLPP is a bona fide tumor suppressor and (2) whether in the context of *PTEN* loss, loss of PHLPP bypasses senescence (similar to PP2a targeting) or triggers a senescence response in vivo (similar to complete PTEN loss). We have gathered strong evidence supporting a tumor-suppressive role for the *PHLPP* gene in human cancers of various tissues. To validate its role in vivo, we are crossing *Phlpp* null mice with *Pten* mutant mice. This analysis will allow us to define tissues where *Phlpp* is essential for tumor suppression and answer the question of whether combined loss of these two major Akt antagonists triggers senescence.

ROLE OF PTEN LEVELS IN CANCER INITIATION AND TREATMENT

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:* 385–396 [2003]), whereas complete *Pten* loss was shown to prevent tumorigenesis

through the p53-dependent senescence response until p53 mutation paves the way for full-blown cancer. Accordingly, prostates of mice harboring partial *Pten* loss spontaneously degrade Pten to allow formation of prostate cancer. Yet, importantly, these lesions do not go on to completely lose Pten at the gene or protein level, a compelling illustration of their ability to maximize proliferation while avoiding the senescence response caused by complete *Pten* loss. Our analysis of human prostate cancer specimens also confirms frequent retention of clearly reduced PTEN levels. Therefore, by using mouse models with partial *Pten* loss, we found that spontaneous and subtle further reduction of Pten levels triggers activation of the downstream oncogenic kinase Akt, illustrating that a threshold must be crossed for initiation of cancer.

It is important to note that such spontaneous down-regulation of Pten is never observed in the prostate of wild-type animals, sometimes observed in the $Pten^{+/-}$ prostate (30% of mice), and is always occurring in $Pten^{hy/-}$ animals (mice with one-fourth 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 whether restoration of PTEN might not just antagonize but also revert tumor growth, analogous to the concept of oncogene addiction.

To address this issue experimentally, we have designed reversibly inducible short-hairpin-based microRNAs in collaboration with the labs of Drs. Lowe and Hannon here at CSHL. As shown by Dr. Lowe's recent work, such hairpin designs can be used in vivo for reversible knockdown, which permits investigation of the therapeutic value of tumor suppressor restoration. Through our approach, we will knock down Pten levels in mouse prostate until tumors form analogous to the models mentioned above. At this time, hairpin activity will be blocked, Pten restored, and tumor volume monitored for regression using magnetic resonance imaging (MRI) methods. Of the 250,000 American men diagnosed with prostate cancer each year, approximately half will present with partial *PTEN* loss in their lesions, and thus, we expect our results to be of great importance in establishing treatment modalities that interfere with disease progression via PTEN stabilization.

MOLECULAR MECHANISMS OF PTEN REGULATION IN CANCER

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 post-translational cellular response (enhanced degradation) cooperate 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 approaches that can be expanded to in vivo analysis.

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RAS AND RHO GTPASES AND THE CONTROL OF SIGNAL TRANSDUCTION

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Research in my laboratory has focused on the role of the Ras and Rho GTPase family members in signal transduction. Ras and Rho family members have key roles in cellular activities controlling cell growth control, differentiation, and morphogenesis. Alterations in Ras and Rho functions have been shown to result in several disease processes, including cancer and neurological 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 neurological disorders. Below are highlights of the main projects that have been carried out during the past year.

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. Increasing evidence points to a critical role for Rap1 in the control of epithelial morphogenesis, and recent studies suggest a dysregulation of Rap1 signaling in malignant processes. How Rap1 proteins exert their effects in vivo has remained largely elusive. We previously described the identification of the mammalian junctional protein AF-6 as a Rap1-interacting protein. To demonstrate the physiological relevance of this interaction, we decided to carry out functional analyses of their orthologs (Rap1 and Canoe) in the more genetically tractable *Drosophila* system. We found that both Rap1 and Canoe are required for epithelial migration events in the embryo and that Canoe acts as a downstream effector of Rap1 in these processes.

More recently, we identified a *Drosophila* Rap1-specific exchange factor, dPDZ-GEF, which is responsible for Rap1 activation in migrating embryonic epithelia. *Canoe* (*cno*) acts downstream from *Rap1* and *dPDZ-GEF* in this event. We demonstrated that dPDZ-GEF/Rap1/Canoe signaling modulates cell shape and apicolateral cell constriction in embryonic and wing disc epithelia. Interestingly, our data indicate that dPDZ-GEF signaling is linked to myosin II function. Both *dPDZ-GEF* and *cno* show strong genetic interactions with the myosin II-encoding gene, and myosin II distribution is severely perturbed in epithelia of both mutants. These findings provide first insight into the molecular machinery targeted by Rap signaling to modulate cell shape in epithelial morphogenesis at different developmental stages.

ROLE OF DOK PROTEINS IN MITOGENIC AND ONCOGENIC SIGNALING

Dok-1 (also called $p62^{dok}$) was initially identified as a tyrosine-phosporylated 62-kD protein associated with Ras-GAP in Ph^+ chronic myeloid leukemia (CML) blasts and in v-Abl-transformed B cells. This protein was termed Dok (downstream of kinases), since it was also found to be a common substrate of many receptor and cytoplasmic tyrosine kinases. Subsequently, six additional Dok family members have been identified. 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 $p210^{bcr\text{-}abl}$. Strikingly, in collaboration with Dr. Pandolfi's group (Memorial Sloan-Kettering Cancer Center), we also found that mice lacking both *Dok-1* and *Dok-2* spontaneously develop a CML-like myeloproliferative disease, likely resulting from increased cellular proliferation and reduced apoptosis. Thus, Dok proteins function as negative regulators of mitogenic and oncogenic signaling.

To further delineate the function of Dok-1 in oncogenic signaling, we examined the expression of Dok-1 protein in cells expressing various oncogene tyrosine kinases. Interestingly, we observed that $p210^{bcr\text{-}abl}$, v-Abl, and oncogenic forms of Src induce down-regulation of Dok-1 expression in a number of cells lines and that the reduction in the levels of Dok-1 protein requires tyrosine kinase activity of these oncogenes. Inactivation of tumor suppressors is an event that is observed in numerous tumors. Since the inactivation of the Dok-1 gene acceler-

ates the progression of CML in mouse models, whereas overexpression of Dok-1 dramatically inhibits the proliferation of $p210^{bcr\text{-}abl}$-expressing cells as well as Src-induced transformation, our current findings suggest that oncogene-induced down-regulation of Dok-1 might be an event that contributes to the progression of tumorigenesis.

ROLE OF RHO REGULATORS IN NEURONAL DEVELOPMENT AND DISORDERS

Accumulating data indicate that the Rho family of GTPases (including Rac, RhoA, and Cdc42) and their regulatory molecules have critical roles in many aspects of neuronal development and function. Their importance in these processes is further emphasized by the findings that mutations in Rho-linked genes are associated with neurological disorders and defects in neuronal development. The cellular and molecular mechanisms by which aberrations in Rho signaling lead to such deficits remain largely elusive. During the past year, we have focused on the functional characterization of two upstream regulators of the Rho GTPases in neuronal development and function, namely, oligophrenin-1 and DOCK7.

Oligophrenin-1 (*OPHN1*), a gene located on chromosome Xq12, codes for a negative regulator of Rho GTPases. Mutations in *OPHN1* (which result in OPHN1 loss of function) have been reported in families with mental retardation (MR) associated with epilepsy and/or cerebellar hypoplasia. Abnormal behavior, impaired language skills, and motor development delays were noted for several of these patients. Interestingly, similar deficits have been observed in patients with autism. Until recently, the function of OPHN1 in the brain remained unknown. We demonstrated that OPHN1 is present in neurons of major regions of the brain, including the hippocampus, cerebellum, and cortex. Using antisense and RNA interference (RNAi) approaches, we found that OPHN1 has a critical role in spine morphogenesis of CA1 pyramidal neurons in hippocampal slices. We also demonstrated a biochemical interaction between OPHN1 and Homer, a postsynaptic adaptor molecule involved in spine morphogenesis and synaptic transmission. Significantly, we more recently obtained evidence that appropriate OPHN1 signaling is required for developmental and activity-dependent modification of synaptic strength at CA3-CA1 synapses of the hippocampus. Together, our data suggest a mechanism by which mutations in *OPHN1* can contribute to the cognitive deficits observed in the *OPHN1* patients.

The second protein, DOCK7, is a novel activator that we identified for Rac GTPases and it belongs to the DOCK180-related family. 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 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 lead to phosphorylation and inactivation of the microtubule-destabilizing protein stathmin/Op18 in the nascent axon and that this event is important for axon development. Our findings unveil 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 set out to monitor neuronal polarization during the migration of newborn developing neurons within an intact brain structure and to establish DOCK7's role in this process. In particular, we implemented the in utero electroporation method that enables highly efficient introduction of vectors coexpressing proteins or hairpin RNAs of interest with a fluorescent protein marker (e.g., enhanced green fluorescent protein [EGFP] or t-dimer Tomato) into ventricular zone (VZ) cells in embryonic cerebral cortices. Interestingly, our preliminary data suggest that DOCK7 knockdown leads to inhibition of normal cortical migration. We are currently further determining the role of DOCK7 in the polarization and migration of cortical neurons in brain slices and are also assessing its involvement in the differentiation of living cerebellar granule cells. Together, these studies will contribute to a better understanding of the determinants/events involved in neuronal polarization and differentiation and the role of DOCK7 in these important processes.

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NEUROSCIENCE

Using a combination of advanced theoretical and experimental approaches, neuroscientists at CSHL seek to elucidate fundamental properties and mechanisms underlying key aspects of brain function, as well as the working brain's system properties, manifest in the design and logic of neural circuitry. Basic science discoveries are being applied, at the same time, in studies of brain and neural system malfunction in illnesses such as schizophrenia, autism, Parkinson's disease, and Alzheimer's disease, which afflict millions.

The focus of research in Grigori Enikolopov's lab is on stem cells in the adult brain and the relationship between the generation of new nerve cells, or neurogenesis, and mood disorders. They have generated several models to study how stem cells give rise to neural progenitor cells (NPCs) and, ultimately, to neurons. They are using these models to determine the targets of antidepressant therapies, identify signaling pathways that control neurogenesis, and search for neuronal and neuroendocrine circuits involved in mood regulation. This year, the lab was part of a team that validated the first biomarker that permits NPCs to be tracked, noninvasively, in the brains of living human subjects. This could help reveal how neurogenesis is related to the course of diseases such as depression, bipolar disorder, Alzheimer's, Parkinson's, and multiple sclerosis.

Jonathan Sebat's laboratory is studying the role of genetic variation and, particularly, gene-copy-number variation in autism and schizophrenia. This year, they published findings of a research collaboration with Michael Wigler's lab that indicated spontaneous mutations—gene mutations in children that do not occur in either parent—are far more common in autism than previously thought. They are now trying to identify which mutations occur in the largest fraction of cases and how each one causes perturbations that lead to the autism phenotype. Is there a common biological process or pathway in which some or all of the identified genes participate? Evidence suggests that the genes in question are rare high-penetrance mutations and that they are connected within biological networks. Sebat and colleagues have embarked on a study of schizophrenia that similarly seeks to discover the importance of spontaneous mutation in causality.

Roberto Malinow's lab is investigating how neurons communicate and how that communication can be modified, under normal and pathological conditions. In their extensive work on synapses in the rodent brain, they have, for instance, monitored the movement of receptor molecules into synapses and have demonstrated, in vivo, that this form of plasticity is associated with learning. Malinow's team is determining the molecular mechanisms by which NMDA- and AMPA-type receptors are delivered to and removed from synapses. Their work suggests that an increase in synaptic AMPA receptors could be the molecular basis of memory storage, whereas an increase in NMDA receptors may control the ease with which such modifications take place. The lab also applies its knowledge about synapses to the study of Alzheimer's disease, and specifically the question of how β-amyloid causes the loss of AMPA receptors and the breakdown of synapses.

Hiro Furukawa's lab is studying synaptic transmission at the molecular level. They focus on the NMDA receptor, a very large molecule whose three-dimensional atomic structure the group has undertaken to solve. They are especially interested in the structure of the so-called ligand-binding domain, the site at which neurotransmitters dock with the receptor. There are at least four distinct NMDA receptor subtypes, which are expressed differentially in different parts of the brain at different stages of life. Furukawa's team seeks to understand the pharmacological specificity of ligand binding in NR2A and NR2D subtypes, in order to provide a blueprint for future drug design. The lab is also studying how risk factors for Alzheimer's disease, such as the APOE lipoprotein, associate with NMDA receptors.

Since biological mechanisms of memory are highly conserved through evolution, many features of memory in higher organisms can be studied in simpler ones, such as fruit flies, that are easy to manipulate. Josh Dubnau and colleagues are using flies to study memory. They seek to identify genes and gene pathways important for memory and likely to be conserved across phyla. Those shown to be conserved

take on importance in research on human pathologies such as mental retardation and Alzheimer's disease. They also seek to understand how the neural circuitry of the fly brain works. In an experiment in what Dubnau calls artificial evolution, they hope to learn how higher-order networks of fly genes interact in the formation of memory.

The Zador, Mainen, and Kepecs labs contribute to CSHL's Center for the Neural Mechanisms of Cognition (CNMC), which develops rodent models that combine behavioral training with electrophysiological recording to understand the neurological basis of higher brain functions such as learning, memory, motivation, and decision-making.

Anthony Zador's lab investigates how the cortex processes sound, how that processing is modulated by attention, and how it is changed by pathology. In the lab's "core assay," the response to sound stimuli of single neurons in the rodent auditory cortex is examined under varying behavioral conditions. Insights obtained are relevant to the study of autism, an illness characterized by the disruption of auditory attention. In animal models of autism, Zador's team is trying to link an inability to screen out background sounds with changes in neural circuits that underlie this disruption. In other work, the lab has challenged the standard model of sound representation in the auditory cortex, which predicts that neural representations of stimuli engage a large fraction of neurons. They have shown that a very small minority of available auditory neurons in a rat cortex react strongly when exposed to a specific sound.

Zach Mainen's lab studies neural coding, or how information is represented in characteristic "spikes" of neuronal electrical discharges. They view this problem in the broader context of neuroeconomics and learning theory: How do animals select appropriate actions on the basis of experience, motivation, and sensory information? Their work in rats has revealed how olfactory information is encoded and transformed into adaptive decisions. Recently, the lab has shed light on the relation of neuronal serotonin release and decision-making in animals and, separately, on an unexpected sensitivity in mammalian brain circuitry deduced from their success in using light-emitting diodes to stimulate tiny subsets of neurons—as few as 60 at a time—to prompt mice to perform a learned behavior.

Adam Kepecs studies neurobiological principles by which the brain makes decisions. He and his colleagues view decisions as elementary units of behavior, from which more complex behaviors are assembled. Yet even simple decisions involve the integration of sensory and memory information with emotional and motivational attributes, requiring the concerted action of millions of neurons across brain regions. Therefore, they take an integrative approach, combining experiments involving controlled rodent behavior with electrophysiology, molecular perturbations, and quantitative analysis. Their current work seeks to elucidate the neurocomputational principles of decision-making, attempting to capture more elusive attributes such as emotion, motivation, or confidence, and neuronal network mechanisms underlying decisions, with particular focus on how specific cell types participate in the neural circuit dynamics of local processing.

Holly Cline's lab is determining the mechanisms by which activity in the brain controls neuronal growth, the generation of synapses, and the development of organized sensory projections between different brain regions. To understand the cellular events responsible for the stabilization of growing neuronal branches, Cline and colleagues have delivered genes of interest into neurons and assessed the effects on synaptic transmission, in nerves that connect the retina and a part of the brain called the tectum involved in visual processing. In other experiments, they have obtained time-lapse imagery indicating that neurons grow by a counterintuitive process: Many new branches are added to neurons, but the majority are rapidly retracted. Cline has learned that visual experience is a key modifier in how these circuits are built.

Yi Zhong's lab studies the neural basis of learning and memory. They work with fruit fly models to study genes involved in human cognitive disorders, including neurofibromatosis and Noonan's Syndrome. Mutations of the neurofibromatosis 1 (*NF1*) gene cause learning defects and neurofibromas—nerve-sheath tumors that split nerve fibers apart. The lab's analyses of *Drosophila NF1* mutants have revealed how expression of the mutant gene affects a pathway crucial for learning. Further studies aim to reveal mechanisms of pathology. This year, the lab discovered that the *NF1* gene and the gene (called *corkscrew*) causally implicated in Noonan's share a biochemical pathway. Zhong and colleagues are also

examining mechanisms of learning and memory at cellular and network levels in the *Drosophila* brain. Their current focus is olfactory-related associative learning.

By comparing mechanisms of learning and memory in fruit flies, humans, and other organisms, Tim Tully and colleagues have revealed ancient molecular pathways of memory conserved by evolution. They have identified "candidate memory genes" in flies, which they are systematically disrupting, in vivo, to confirm behaviorally their affect on memory formation. The lab demonstrated this year that NMDA-receptor-dependent long-term memory in flies occurs not only in the mushroom body, but also in a structure called the ellipsoid body not previously implicated in memory formation. In these and other efforts, the lab seeks a comprehensive genetic etiology of memory that will ultimately lead to more effective therapies for memory loss.

Josh Huang and colleagues study how the brain develops neural networks that use GABA, the brain's primary inhibitory neurotransmitter. Their work has implications in illness such as autism and schizophrenia, which involve the altered development and function of GABAergic circuits. They have shown that the maturation of GABAergic connections in the visual cortex is regulated by sensory experience during a postnatal critical period. This year, they reported a surprising finding about GABA itself. It has long been known that GABA binds to specific neuronal transmembrane receptors, precipitating a change in membrane voltage. They now find that GABA also serves as a trophic factor, impacting signaling that determines whether a synapse is stable and whether axons will grow or retract.

Dmitri Chklovskii and colleagues are mapping the "wiring" of the rat brain using electron microscopy. Images of extremely thin brain slices are "stacked" by a computer to assemble a three-dimensional brain rendering. Even in a small volume, 1000 times thinner than a human hair, there are roughly 1000 axons and 100 dendrites. The spatial organization, or "packing," of axons and dendrites is random. Yet, the junction points at which axons and dendrites of different neurons exchange signals, called synapses, may form based on a definite logic. The lab is expanding the area covered by their mapping project, which, Chklovskii observes, is prelude to the larger aim of modeling the electrical activity of neuronal networks.

Alexi Koulakov and colleagues are trying to discover the mathematical rules by which the developing brain assembles itself, with particular focus on the formation of sensory circuits such as those involved in visual perception and olfaction. The visual system of the mouse is well-understood, in neuroanatomical terms. 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 maturity, enables the individual to distinguish one smell from another with astonishing specificity and to remember such distinctions over time.

Partha Mitra seeks to develop an integrative picture of the brain, incorporating theory, informatics, and experimental work. His theoretical interests are primarily in formalizing the treatment of biological function using ideas and methods from engineering. In informatics work, his lab is developing computational tools for analyzing neurobiological data, particularly electrophysiological data from experiments that probe cognitive phenomena. Mitra has organized the Brain Architecture Project, a multi-institutional effort to curate information from the literature about human neuroanatomical connectivity. In their experimental research, Mitra and colleagues, in collaboration with Josh Dubnau, study memory formation in the fruit fly. The lab also studies the evolution of song culture in zebra finches.

CSHL Fellow Gilbert L. Henry studies cellular differentiation using the vertebrate taste bud as a model. Taste buds comprise unspecialized "progenitor stem cells" at their base and periphery. These cells divide and their daughters become specialized receptor cells that migrate toward the center and apex of the taste bud. Different taste receptor cells respond to different tastes and each forms a number of synapses on neurons that transmit information regarding the chemical nature of food to the brain. Henry is working to define both taste receptor and taste progenitor cells at molecular resolution.

FROM NEURONAL CIRCUIT RECONSTRUCTIONS TO PRINCIPLES OF BRAIN DESIGN

D. Chklovskii B. Chen Y. Mishchenko
A. Genkin Q. Wen

How does electrical activity in neuronal circuits give rise to intelligent behavior? We believe that this question is impossible to answer without a comprehensive description of the connectivity in neuronal networks. Such a description may be a wiring diagram, which catalogs all neurons and synaptic connections between them. In collaboration with several laboratories, we are reconstructing vertebrate and invertebrate wiring diagrams from electrophysiological, light, and electron microscopical data. To gain insight into brain function from the wiring diagrams, we formulate engineering principles of brain design and test them experimentally. By focusing initially on explaining the function of simpler organisms, we are assembling a theoretical framework and accumulating experience necessary to understand more complex systems, such as the mammalian neocortex.

STATISTICAL CIRCUIT RECONSTRUCTIONS FROM LIGHT MICROSCOPY AND ELECTROPHYSIOLOGY

We have developed the necessary theoretical framework to estimate, or reconstruct statistically, wiring diagrams from the shapes of axonal and dendritic arbors visualized with light microscopy. Such reconstructions are based on the principle that physical proximity between the axons of one neuron and the dendrites of another predicts probabilistically synaptic connections between the two. This principle is not new. However, only with recent developments in cell labeling, imaging, and computing infrastructure has the comprehensive geometric description of neuronal connectivity become an attainable goal. As a demonstration, we have statistically reconstructed the cortical column from three-dimensional shapes of dozens of neurons labeled in vivo.

To determine how well geometric connectivity maps correspond to functional connectivity, we compared maps calculated from neuronal shapes with those obtained electrophysiologically in collaboration with Karel Svoboda's laboratory here at CSHL. In many cases, our maps accurately predicted connectivity on the level of populations of neurons, i.e., projections between cortical layers.

A geometric connectivity map is a particularly appropriate description of cortical circuits in the face of ongoing changes in connectivity. Previously, we proposed that the growth and retraction of dendritic spines could alter connectivity, and Svoboda's laboratory has observed such experience-dependent plasticity in adult animals. As geometric connectivity maps rely on the mostly stable layout of axons and dendrites, rather than on the more volatile spines, they provide an invariant description of cortical circuits.

Geometric maps have their limitations: They yield the probability of connections but cannot predict for certain whether a given pair of neurons is connected. The maps also do not capture the strong correlations we found in synaptic connections in pairs and triplets of neurons when we analyzed electrophysiological recordings by collaborators.

COMPLETE CIRCUIT RECONSTRUCTIONS FROM ELECTRON MICROSCOPY

Electron microscopy is currently the only technique capable of reconstructing wiring diagrams in their entirety. By detecting synapses on electron micrographs of serial sections and tracing axons and dendrites to their somas, one can identify synaptically connected neurons. The largest such reconstruction, the *Caenorhabditis elegans* nervous system, was carried out manually at the Medical Research Council (MRC), Cambridge, England, and finalized by us.

Complete electron microscopic circuit reconstruction is a painstakingly slow process because of the sheer amount of data involved. Axons and dendrites spanning millimeters must be traced with a resolution of a few nanometers. The reconstruction of the *C. elegans* nervous system, containing only 279 neurons, took the equivalent of several decades of work to complete!

To reconstruct more complex circuits, we are using techniques from image processing and machine learning to develop automated tracing algorithms. So far, we have reconstructed a neuropil volume of 1000 cubic microns. We intend to scale up our automated algorithms to fully reconstruct circuits of wide interest

among neurobiologists, such as the fly brain, the vertebrate retina, and the cortical column.

FROM WIRING DIAGRAMS TO BEHAVIOR

Armed with wiring diagrams, we are starting to understand how electrical activity in neuronal circuits generates behavior. Our initial strategy is to focus on relatively simple circuits and behaviors. Once those are well understood, our experience will serve as a foundation for reverse engineering more complex systems.

One such simple system is the neuronal control of undulatory locomotion in *C. elegans*. Many species rely on a central pattern generator for undulatory locomotion. The *C. elegans* wiring diagram by itself does not support required oscillations. This implies that undulatory locomotion likely relies on propriosensory feedback. To understand how locomotion arises, we combine measurements of body muscle activity using a calcium indicator and a simple model based on the known wiring diagram.

This simple system demonstrates that even the complete wiring diagram is not sufficient to understand how a circuit works. Modeling circuit function requires knowing certain dynamic parameters of neurons and synapses, most of which are missing from the wiring diagrams. The problem goes even deeper: Not only are most of the parameters missing, but we do not even know *which* of them are essential and which are not.

DESIGN PRINCIPLES BASED ON OPTIMIZATION THEORY

For models of brain function to have predictive power, an appropriate level of abstraction must be chosen and the essential parameters must be identified and measured. When we look to disciplines where modeling is well established, such as physics or engineering, we find that model building must be based on fundamental principles, or physical laws, such as the laws of conservation. Identifying similar fundamental principles in biology would help constrain the choice of possible models and focus our efforts.

As biological systems have evolved over hundreds of millions of years, their design has been optimized under various physical constraints. It is thus natural that a theoretical approach to biology must incorporate aspects of constrained optimization. We quantitatively formulate principles of brain design and rely on optimization theory to answer *why* questions.

A particularly successful example of optimization in biology is the wiring economy principle proposed by Ramón y Cajal more than 100 years ago. By quantitatively formulating and applying this principle, we were able to make unexpected, experimentally testable predictions and explain many aspects of brain design, such as the existence and structure of cortical maps, the spatial arrangement of neurons, the existence of dendritic spines, the dimensions of axons and dendrites, and the segregation of the neocortex into the gray and white matter. In addition to the many successes of our theoretical predictions, experiments have revealed several discrepancies. These discrepancies led to the discovery of another important principle of brain design: maximization of entropy (or information capacity). This principle in turn explains several key properties of synapses and the shapes of neurons.

Our research continues to focus on mapping the brain's structure and establishing its relationship with function. We have reconstructed both statistical and deterministic wiring diagrams and explained many structural observations as outcomes of constrained optimization. We plan to reconstruct wiring diagrams of more complex circuits and understand their function by combining anatomical, physiological, and theoretical approaches.

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In Press

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BRAIN DEVELOPMENT AND PLASTICITY

H. Cline J. Bestman, K. Bronson, K. Burgos, S.-L. Chiu, J.S. da Silva, J. Demas, R. Ewald, M. Hiramoto, S.-Y. Lee, J. Lee-Osbourne, H. Li, J. Li, L. Schiapparelli, P. Sharma, W. Shen, V. Thirumala, R.-Y. Tzeng, K. Van Keuren-Jensen

Our research is focused on understanding the mechanisms by which experience controls the development of the brain. We address this fundamental question by examining the development of the visual system in *Xenopus* tadpoles and the development of the spinal cord in zebra fish. The visual system of *Xenopus* is well-known for its experience-dependent plasticity. We have established this preparation as an excellent experimental system in which to conduct in vivo time-lapse imaging studies of neuronal development and synaptogenesis, combined with both gene transfer and electrophysiological studies of visual system function.

During the past 15 years, we have demonstrated a role for afferent coactivity, postsynaptic *N*-methyl-D-aspartate receptor (NMDAR) activity, and downstream activation of calcium-dependent enzymes including calcium/calmodulin-dependent protein kinase II (CaMKII) in controlling retinotectal synaptic maturation, optic tectal cell structural plasticity, and topographic map formation (Wu et al., *Science 274:* 972 [1996]; Zou and Cline, *Neuron 16:* 529 [1996]; Wu and Cline, *Science 279:* 222 [1998]; Zou and Cline, *J. Neurosci. 19:* 8909 [1999]; Ruthazer et al., *Science 301:* 66 [2003, 2006]; Haas et al., *Proc. Natl. Acad. Sci. 103:* [2006]).

More recently, we have demonstrated that visual experience has multiple effects on visual system development. A relatively brief period, 4 hours, of visual experience enhances the growth rate of tectal cell dendritic arbors through a mechanism that requires glutamatergic transmission and the RhoA GTPases (Li et al., *Nat. Neurosci. 3: 2*17 [2000]; Li et al., *Neuron 33: 741* [2002]; Sin et al., *Nature 419:* 475 [2002]). This same brief period of visual experience increases the excitability of tectal neurons and their sensitivity to visual stimuli, through a mechanism that requires intracellular polyamines, the modulation of α-amino-3-hydroxy-5-methyl-3-isoazole receptor (AMPA-R) function and compensatory changes in sodium channel activity (Aizenman et al., *Neuron 34:* 623 [2002]; Aizenman et al., *Neuron 39:* 831 [2003]).

The finding that we can use visual stimulation to modify the development and properties of the retinotectal system has spurred our interest in determining the function of activity-induced genes on visual system plasticity. For instance, our studies of Homer, Arc, and CPG15 demonstrate that each has distinct roles in controlling neuronal plasticity. Homer is a widely expressed scaffold protein, which affects calcium signaling and metabotropic glutamate receptor (mGluR) signaling. In addition to finding a role in axon guidance (Foa et al., *Nat. Neuronsci. 4:* 499 [2001]; Foa et al., *J. Comp. Neurol. 487:* 42 [2005]), our more recent work indicates that experience-dependent changes in postsynaptic Homer expression regulates mGluR-mediated plasticity of retinotectal transmission (Van Keuren-Jensen and Cline, *J. Neurosci. 26:* 7575 [2006]). This is particularly interesting in light of recent work suggesting that mGluR-mediated potentiation and depression of synaptic transmission may have a role in developmental neurological disorders such as Fragile X.

CPG15, another activity-induced protein, is noteworthy because it a GPI-linked signaling molecule whose expression results in a large increase in dendritic arbor development, coupled with an increase in glutamatergic retinotectal synaptic maturation and a coordinated elaboration of presynaptic retinal axon arbors (Nedivi et al., *Science 281:* 1863 [1998]; Cantallops et al., *Nat. Neurosci. 3:* 1004 [2000]; Nedivi et al., *J. Comp. Neurol. 435:* 464 [2001]). We have recently shown that CPG15 mediates these changes by promoting synapse formation, which in turn enhances axonal arbor growth (Javaherian and Cline, *Neuron 45:* 505 [2005]). These data suggest that CPG15 is akin to an activity-induced targeted growth factor. It now appears that many activity-induced genes function in a homeostatic manner to maintain synaptic strength within a functional operating range, despite experience-dependent increases or decreases in synaptic strength. This is the case for Arc (Rial Verde et al., *Neuron 52: 461* [2006]) and Homer, as well as ornithine decarboxylase, which generates polyamines and thereby regulates neuronal excitability and the strength of glutamatergic synaptic transmission.

Enhanced Visual Activity In Vivo Forms Nascent Synapses in the Developing Retinotectal Projection

C.D. Aizenman

Patterned neural activity during development is critical for proper wiring of sensory circuits. Previous work demonstrated that exposing freely swimming *Xenopus* tadpoles to 4 hours of enhanced visual stimulation accelerates the dendritic growth rate of optic tectal neurons in vivo (Sin et al., *Nature 419:* 475–480 [2002]). Here, we test whether this same period of visual stimulation increased synaptic maturation and formation of new synapses in the retinotectal pathway.

We assessed synaptic properties of stage-48 tadpoles that were exposed to a simulated-motion stimulus for 4–5 hours. On the basis of our findings that immature retinotectal synapses have greater paired pulse facilitation compared to more mature synapses, consistent with a lower release probability (Pr), we used a paired pulse protocol to elicit responses selectively from nascent synapses with low Pr. Although AMPA:NMDA ratios for single and paired stimuli were the same in control tadpoles, visual stimulation caused a relative decrease in the AMPA:NMDA ratio of the paired response. We evoked retinotectal synaptic transmission in the presence of Sr^{2+} to record asynchronous vesicle release. We compared evoked mEPSCs (excitatory synaptic currents) induced by single and paired stimuli and found that visual stimulation selectively enhances the amplitude and number of AMPAR-mediated mEPSCs evoked by paired stimuli relative to those evoked by single stimuli.

Together, these results show that enhanced visual stimulation affects both AMPAR- and NMDAR-mediated responses in a population of synapses revealed by paired-pulse stimulation. This suggests that in vivo visual stimulation increases synapses that have a low Pr and that have properties consistent with immature synapses.

Rapid Activity-dependent Delivery of the Neurotrophic Protein CPG15 to the Axon Surface of Neurons in Intact *Xenopus* Tadpoles

I. Cantallops

CPG15 (also known as Neuritin) is an activity-induced GPI-anchored axonal protein that promotes dendritic and axonal growth, and accelerates synaptic maturation in vivo. Here, we show that CPG15 is distributed inside axons and on the axon surface. CPG15 is trafficked to and from the axonal surface by membrane depolarization. To assess CPG15 trafficking in vivo, we expressed a ecliptic pHluorin (EP)-CPG15 fusion protein in optic tectal explants and in retinal ganglion cells of intact *Xenopus* tadpoles. Depolarization by KCl increased EP-CPG15 fluorescence on axons. Intraocular kainic acid (KA) injection rapidly increased cell surface EP-CPG15 in retinotectal axons, but coinjection of TTX and KA did not. Consistent with this, we find that intracellular CPG15 is localized to vesicles and endosomes in presynaptic terminals and colocalizes with synaptic vesicle proteins. The results indicate that the delivery of the neurotrophic protein CPG15 to the axon surface can be regulated on a rapid timescale by activity-dependent mechanisms in vivo.

Refining the Roles of GABAergic Signaling during Neural Circuit Formation

C.J. Akerman

Our understanding of the role of GABA signaling in circuit development is rapidly expanding. We review three recent refinements in our understanding of the diverse roles that GABA has at different stages of neural circuit formation. Recent evidence has shown that

1. Depolarizing GABA has at least a permissive role in promoting both excitatory and inhibitory synaptogenesis in developing neurons (including newly generated neurons in the adult).
2. GABAergic circuits sculpt the temporal and spatial aspects of synaptic integration. Consequently, early developmental events affecting the establishment of GABAergic circuits will control subsequent activity-dependent refinements of information processing and circuit function.
3. The molecular mechanisms by which GABAergic signaling has a role in the regulation of the balance between GABAergic and glutamatergic transmission in developing circuits. We concentrate on the effects of the signaling by $GABA_A$ receptors, as told from the point of view of the GABA-responsive cells, and do not discuss mechanisms that govern GABA release or activity of GABAergic neurons per se.

Regulation of Dendritic Arbor Development and Plasticity by Glutamatergic Synaptic Input: A Review of the Synaptotrophic Hypothesis

K. Haas

The synaptotropic hypothesis, which states that synaptic inputs control the elaboration of dendritic (and axonal) arbors was articulated by Vaughn in 1989. Today, the role of synaptic inputs in controlling neuronal structural development remains an area of intense research activity. Several recent studies have applied modern molecular genetic imaging and electrophysiological methods to this question and now provide strong evidence that maturation of excitatory synaptic inputs is required for the development of neuronal structure in the intact brain. Nevertheless, some studies fail to show evidence that afferent inputs affect structural development. We review data that both support and refute the hypothesis with the expectation that understanding the circumstances when the data do and do not support the hypothesis will be most valuable.

NR2A and NR2B Have Distinct but Overlapping Roles in Shaping the Development of Dendritic Arbor Morphology In Vivo

R.C. Ewald, K.R. Van Keuren-Jensen, C.D. Aizenman

NMDARs are important for neuronal development and circuit formation. The NMDAR subunits NR2A and NR2B are biophysically distinct and differentially expressed during development, but their individual contribution to structural plasticity is unknown. Here, we test whether NR2A and NR2B subunits have specific functions in the morphological development of tectal neurons in living *Xenopus* tadpoles. Exogenous subunit expression and endogenous subunit knockdown shifts the synaptic NMDAR composition toward NR2A or NR2B, as shown electrophysiologically. We analyzed the dendritic arbor structure and found evidence for both overlapping and distinct functions of NR2A and NR2B in dendritic development. Control neurons obtain regions of high local branch density in their dendritic arbor, whereas exogenous expression of either NR2A or NR2B decreases local branch clusters, indicating a requirement for both subunits in dendritic arbor development. Knockdown of endogenous NR2A, however, reduces local branch clusters compared to knockdown of NR2B, suggesting more complex NR2 signaling.

Analysis of the underlying branch dynamics shows that exogenous NR2B-expressing neurons are more dynamic than control or exogenous NR2A-expressing neurons, demonstrating subunit-specific regulation of branch dynamics. However, visual experience-dependent increases in dendritic arbor growth rate seen in control neurons are blocked in both exogenous NR2A- and NR2B-expressing neurons. Furthermore, AMPAR-mediated synaptic transmission is decreased in neurons with knocked-down or exogenous NMDAR composition, indicating overlapping roles for NR2A and NR2B in regulating synaptic transmission. Thus, we show that NR2A and NR2B have subunit-specific properties in dendritic arbor development, but also overlapping functions, indicating a requirement for both subunits in neuronal development.

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GENETICS OF MEMORY IN *DROSOPHILA*

J. Dubnau A. Blum W. Li
M. Cressy H. Qin
C. Jurgensen

The long-term goal of our research is to understand memory. Dissection of complex behaviors such as memory and learning will require a multidisciplinary approach that will include discovery and manipulation of the relevant genetic, cellular, and anatomical pathways, as well as computational modeling of how information is processed in the brain. Work in genetic model systems such as *Drosophila* can contribute to our understanding in several ways. First, by enabling discovery of genes and genetic pathways underlying behavior, such model systems provide entry points for dissection of cellular mechanisms that are often conserved. Second, systematic manipulation of gene function in relevant anatomical loci of the brain allows a conceptual integration of findings from cellular and behavioral neuroscience.

Our gene discovery efforts already have identified a role in memory for highly conserved pathway(s) involved with subcellular control of translational regulation. Many of these molecules have counterparts in vertebrates that also appear to have important roles in brain function and, in some cases, may be linked to human cognitive dysfunction. Our genetic investigations also provide insight to investigate the neural circuitry relevant to memory because gene expression patterns often suggest hypotheses that can be tested by spatially restricted genetic manipulations. Here, our recent findings demonstrate that short-term and long-term memory are supported by anatomically distinct memory traces. This appears to reflect a fundamental (but poorly understood) feature of memory formation. At the behavioral level, we are uncovering conserved behavioral properties of learned fear that may be relevant to fear disorders in humans.

Discovery of Genetic Pathways Involved with Memory

J. Dubnau, C. Jurgensen, W. Li [in collaboration with T. Tully, C. Zhang, and M. Zhang, Cold Spring Harbor Laboratory; F. Li, Tsinghua University]

We have used two expression-profiling methods to identify transcriptional responses during memory consolidation. First, we used Afymetrix arrays to compare expression profiles after spaced training, which induces both short-term and protein-synthesis-dependent long-term memory and massed training, which only induces short-lived memory (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). We thus identified a large number of candidate memory genes differentially expressed at three different retention intervals after spaced versus massed training. Using real-time polymerase chain reaction (PCR) follow-up assays, we confirmed differential expression for nearly 60 of these transcripts. As a second complementary approach, we have recently used chromatin immunoprecipitation of posttranslationally modified histones, followed by hybridization of the associated DNA with tiling arrays. This approach has led to the identification of an overlapping set of transcripts with the expression profiling. The points of overlap between these two approaches and results of a forward mutagenesis screen (Dubnau et al., *Curr. Biol. 13:* 286 [2003]) should identify genetic pathways that are critical to memory formation.

Each candidate memory gene (CMG) identified by the above microarray approaches serves as an entry point for molecular genetic investigation of gene function in memory. These CMGs then become fodder for in vivo genetic manipulations to forge mechanistic connections between individual gene pathways and memory formation. With that aim in mind, we are focusing on subcellular control of neuronal translation, one of several pathways suggested from the array experiments, as well as from a forward mutagenesis screen (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). In humans, cellular mechanisms of dendritic translation is of relevance to the etiology of fragile-X mental retardation syndrome. Our genetic studies in *Drosophila* already support a role in memory for several components of this cellular machinery. These include *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. A large number of the known components of the mRNA localization machinery also were identified in our various screens, as well as of the apparatus for regulating cytoplasmic polyadenylation-regulated translational control. Importantly, many genetic reagents to manipulate these pathways are extant in *Drosophila*.

Synaptic Targets of Pumilio

J. Dubnau, W. Li [in collaboration with G. Chen, A. Krainer, T. Tully, Q.S. Zhang, M. Zhang, Cold Spring Harbor Laboratory]

Drosophila Pumilio (Pum) protein is a translational regulator involved in embryonic patterning and germ-line development. Recent findings demonstrate that Pum also has an important role in the nervous system, both at the neuromuscular junction (NMJ) and in long-term memory formation (Dubnau et al., *Curr. Biol. 13:* 286 [2003]). In neurons, Pum 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 Pum in the nervous system remain largely unknown in any species. We hypothesized that Pum 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 to predict Pum targets among mRNAs whose products have synaptic localization. We then used both in vitro binding and two in vivo assays to functionally confirm the fidelity of this informatics screening method. We find that Pum 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 (Fig. 1). Finally, we show that the endogenous *dlg1* mRNA can be regulated by Pumilio in a neuronal context, the adult mushroom bodies (MB), which is an anatomical site of memory storage (Fig. 2). Together, these findings identify a PSD95 ortholog as a major neuronal target of Pumilio.

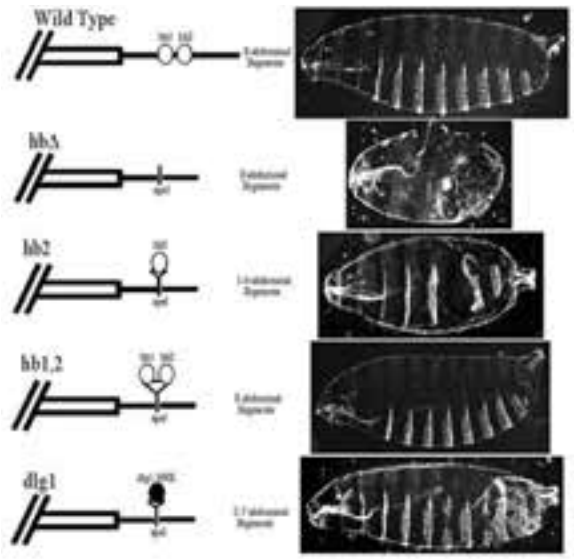

FIGURE 1 In vivo confirmation of predicted NRE elements. Cartoon representations of the *hb*-transgene constructs and representative examples from cuticle preparations of corresponding transformant lines, showing normal/abnormal or rescued/partial rescued abdominal segmentation. Transgenic lines containing both *hb* NREs in either the normal context (wild type) or reinserted into the deletion construct (hb1,2) are fully regulated by Pum and yield embryos with the normal complement of eight abdominal segments. Deletion of both of these NREs (hbΔ) prevents Pum-dependent translational repression leading to complete absence of abdominal segmentation. Insertion of either one *hb* NRE (hb2) or the predicted NRE from *dlg1* (dlg1) are each sufficient to partially restore abdominal development.

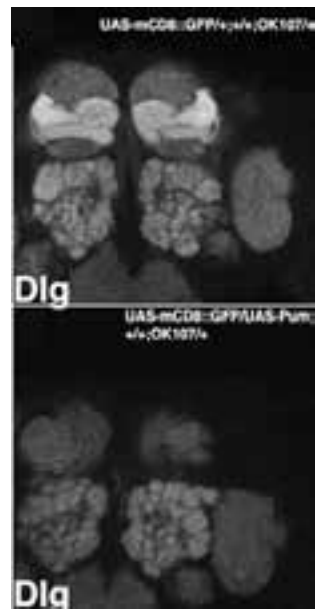

FIGURE 2 Dlg is repressed by overexpression of Pum in MB Kenyon cells. MB expressing Gal4 line (OK107) was used to drive expression of both UAS-mCD8::GFP and UAS-Pum transgenes. Optical sections of the MB lobes. In wild type (*top*), Dlg expression is detected both in MB lobes and AL and in the MB peduncle (not shown). In contrast, Dlg expression is dramatically reduced in the MB lobes (*bottom*) and peduncle (not shown) of Pum overexpressing MBs. AL glomeruli, also stained by Dlg antibody, serve as an internal control.

Functional Anatomy

A. Blum, J. Dubnau

A conserved feature of memory is consolidation through temporally distinct phases such as short-term, middle-term, and long-term memory. Each of the phases can be selectively disrupted by a variety of experimental manipulations, suggesting that they are not only temporally distinct, but also mechanistically distinct. Another conserved characteristic of memory is that the neuronal structures involved with the initial storage are often insufficient or distinct from those involved with long-term memory storage. This remarkable feature of memory has been observed across a wide distribution of species and has been found for both simple Pavlovian associations and more cognitive tasks. But in no case are the underlying cellular or circuit mechanisms understood.

In *Drosophila*, much work has been done to functionally map circuitry required for early phases of memory. It is now well established that olfactory information and the pathways that convey electric shock converge at the MBs, a bilaterally symmetric neuronal structure consisting of approximately 2500 neurons per hemisphere. The intrinsic neurons of the mushroom body, the so-called Kenyon cells, consist of three major subtypes (α/β, α'/β', and γ), each of which have distinct axonal architectures. Together, these three groups of Kenyon cells are believed to be the site of association of multimodal sensory stimuli. This notion is supported by a wide variety of experiments from several labs that demonstrate that a number of different learning mutants can be rescued by transgenic expression restricted to one class or another of Kenyon cell neurons. In particular, *rutabaga* adenylyl-cyclase-dependent cAMP signaling in γ lobe neurons is sufficient to support olfactory short-term memory. In addition, we and other investigators have shown that synaptic output from Kenyon cells is required during retrieval (Dubnau et al., *Curr. Biol. 13:* 286 [2003]; Margulies et al., *Curr. Biol. 15:* 700 [2005]). Together, the data support a model in which initial odor-shock association requires cAMP-dependent plasticity only in MB Kenyon cells and that retrieval requires synaptic output from this structure in order to drive behavioral responses to the odors.

We have conducted the first systematic investigation of the requirements for cAMP signaling in each of the three major Kenyon cell subtypes for each of the temporal phases of memory. We have shown that the classic learning mutant *rutabaga*, a Ca^{2+}-dependent adenylyl cyclase, also has long-lasting memory defects (Fig. 3). By expressing the *rutabaga* cDNA in each of the subsets of Kenyon cell neurons, we have demonstrated that short-term and long-term memory each are supported by *rutabaga* signaling in distinct groups of neurons. The most surprising finding is that short-term and long-term memory can be separately rescued, suggesting that both the initiation and maintenance of these traces is independent.

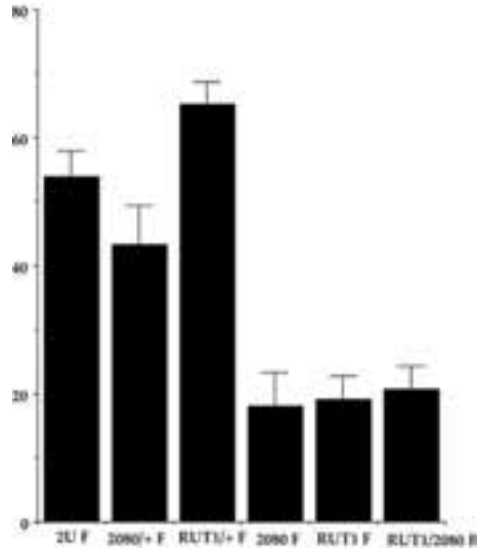

FIGURE 3 *rutabaga* mutants exhibit a severe defect in long-term memory. Memory performance indices 24 hours after repetitive spaced training are shown. Wild type (2U) and animals heterozygous for each of two rutabaga alleles (2080/+ and RUT1/+) each show robust and equivalent levels of long-term memory performance. In contrast, animals homozygous for either allele (2080 or RUT1) or *trans*-heterozygous for the two alleles (RUT1/2080) show severely deficient memory. All data are for females (F) because rutabaga is X-linked. This phenotype has provided the basis for mapping the neural circuits in which rutabaga signaling is required for long-term memory (see text).

Enhancer/Suppressor Screening by Selective Breeding

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

Genetic investigation of memory has revealed that underlying mechanisms are highly conserved across phyla. The cAMP cascade, for example, has been implicated in memory in both invertebrate and vertebrate animals, including humans. The informative power of genetics derives in part not only from identification of genes that influence phenotype, but also from analysis of gene interaction. Saturation mutagenesis for embry-

onic patterning, for instance, identified most of the relevant genes. Equally important, however, were second-site suppressing and enhancing screens. This approach allowed cell-signaling pathways and mechanistic insights to be distilled from what otherwise would be unconnected gene lists.

In the case of complex behavioral phenotypes such as memory, forward mutagenesis has identified a number of relevant genes (Margulies et al., *Curr. Biol. 15:* 700 [2005]). Here too, the most informative instances are where mechanistic interactions among genes are understood. cAMP signaling is the most notable example. Assembly of genes into functional networks, such as the cAMP cascade, is the most challenging aspect of genetics. Although suppressor/enhancer screens have yielded some success, this approach is often not feasible for complex quantitative traits such as memory. Moreover, modifier screens are only designed to detect interactions between pairs of genes, but they generally do not identify more complex gene networks. We are using experimental evolution with identified memory mutants (Dubnau et al., *Curr. Biol. 13:* 286 [2003]) as a strategy to screen for networks of epistasis capable of suppressing the role of cAMP signaling.

Selective breeding of extremes in behavioral phenotypes has a long and fruitful history. In contrast with screening for induced mutations, this strategy normally relies on the presence of preexisting (natural) genetic heterogeneity in a starting population. This method was first used to establish a genetic basis for several complex behavioral traits in a variety of species, including learning ability in *Drosophila*. This approach provided a means to estimate genetic variation in natural populations, to examine the process by which complex traits evolve through selection, and to investigate pleiotropy. Genetic analyses of the selected strains also demonstrated that phenotypic extremes typically derived from interactions among a constellation of alleles. Unlike forward mutagenesis, this quantitative genetic strategy relies on selection of combinations of alleles, thereby placing individual gene function within a network of interactions. This strategy also has the potential to reveal mechanisms underlying evolution of phenotypic variation. In contrast with forward mutagenesis, however, quantitative genetic strategies on their own have not afforded an efficient means to identify and manipulate individual genes. Even with a variety of powerful quantitative-genetic mapping methods (e.g., QTL mapping), molecular identification of underlying loci has been challenging.

Each of the above genetic strategies thus offers complementary strengths for discovery of mechanisms underlying complex behavioral biology. Recent progress in genome-wide sequencing and expression profiling raises the possibility of combining the above methods. There is as yet no case, however, where artificial selection of molecularly identified alleles has been used for memory, no example where the evolution of a phenotype has been followed over successive generations at a mechanistic level, and no study where selection has been used to directly screen for suppression or enhancement of a known signaling pathway. We are using this strategy to identify networks of gene interactions. Our approach relies on artificial selection over the course of multiple generations to "evolve" combinations of known and molecularly tagged gene variants that interact to produce extreme levels of learning.

Extinction Learning: A Model for Posttraumatic Stress Disorder

H. Qin, J. Dubnau

Repeated representation of the conditioned stimulus (CS) without the reinforcement of the unconditioned stimulus (US) results in the diminishment of conditioned responding. This process is called extinction (of Pavlovian conditioning). Extinction was first studied by Pavlov in 1927 and has been reported in both appetitive and aversive paradigms. Extinction is not simply erasing the original CS-US association but appears to be a new learning process that antagonizes the original memory. This type of learning is important biologically, but it also has received attention because of its potential relevance to posttraumatic stress disorder (PTSD), in which an individual continues to fear a particular set of stimuli or a context. This debilitating fear can be highly resistant to extinction.

We are taking advantage of the abundant molecular and genetic tools of *Drosophila melanogaster* to investigate mechanisms of extinction following Pavlovian "fear" learning. We have established a robust assay of extinction and have initiated a focused screen through the extant mutants that affect olfactory memory. We are also using spatially restricted expression of cDNA rescuing constructs to map the circuitry of extinction versus fear learning.

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STEM CELLS, SIGNAL TRANSDUCTION, AND BRAIN FUNCTION

G. Enikolopov J.M. Encinas N. Peunova
T. Michurina J. Tordo
J.-H. Park M. Vidal

Our research is focused on stem cells in the adult organism and signaling molecules that control their fate. Our main focus is on brain stem cells, which retain the ability to produce new neurons throughout adult life, a function that may be important for memory and for mood regulation. We have generated several animal models to track and isolate stem cells and new neurons, and we use these models to study how disease and therapeutic drugs affect stem cells of the brain. Furthermore, we study stem cells of other tissues, looking for commonalities among various types of stem cells on how they decide on whether to stay quiescent, self-renew, or differentiate. We also study the signals that mediate interactions between stem cells and their microenvironment, with particular attention to the regulatory role of a versatile signaling molecule, nitric oxide (NO). Finally, we are trying to translate the knowledge we generate with animal models to human studies.

DEFINED STAGES OF THE NEUROGENIC CASCADE IN THE ADULT HIPPOCAMPUS

New neurons are produced from stem cells through a cascade of events that include symmetric and asymmetric divisions, exit from the cell cycle, and continuous changes of morphology, culminating with the young neuron establishing connections with other cells and becoming integrated into the preexisting neuronal circuitry. Production of new neurons is a dynamic process that responds to a wide range of stimuli which can enhance or suppress neurogenesis and may affect any step of the differentiation cascade.

We aim to define each step of the cascade and to determine the targets of important proneurogenic or antineurogenic stimuli. Our main approach is to generate transgenic reporter animal lines and to use them to dissect the neurogenesis cascade into distinct stages and then identify the steps that are specifically affected by a particular stimulus. We have now combined a new technique of cell labeling that greatly increases the resolution of cell cycle analysis with phenotyping progenitor cell populations. This enabled us to identify all of the major proliferative steps in the life cycle of stem and progenitor cells in the adult hippocampus. It allowed us to present a new scheme of the neuronal differentiation cascade in the hippocampus (in collaboration with Dr. Alex Koulakov here at CSHL) (Fig. 1); this scheme will now serve as a general platform upon which researchers will be able to project the action of any agent that alters neurogenesis. We used this scheme to identify the targets of antidepressant drugs and treatments, exercise, radiation, and chemotherapeutic agents and to address the mechanisms that control the quiescence, self-renewal, differentiation, and death of stem cells in the adult brain.

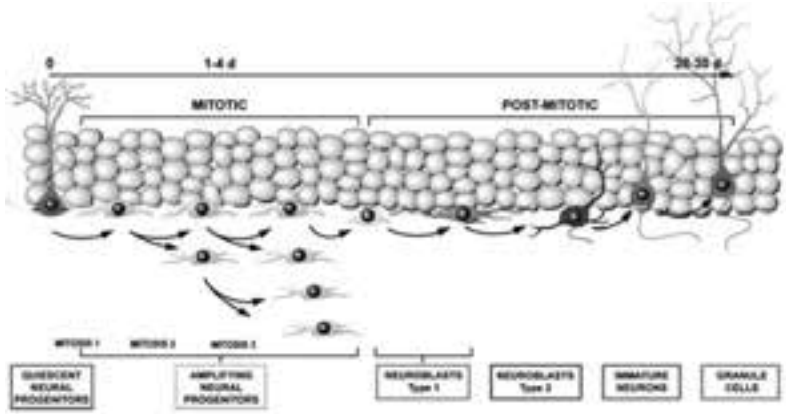

FIGURE 1. A schematic summary of the neuronal differentiation cascade in the hippocampus.

NEUROGENIC TARGETS OF ANTIDEPRESSANT THERAPIES

Emerging evidence indicates a close link between the action of a diverse range of antidepressant therapies and augmented generation of new neurons in the adult hippocampus. Moreover, recent findings suggest that adult hippocampal neurogenesis not only accompanies, but is also required for the behavioral effects of antidepressants (e.g., fluoxetine/Prozac). This discovery may clarify several observations regarding the clinical course of action of antidepressant drugs. For instance, it may explain why an appreciable clinical effect of antidepressants usually takes at least 3–4 weeks: This period may reflect the time required for neural stem cells to proceed through the differentiation cascade and become fully differentiated and integrated neurons.

We used the information on the stages of the neuronal differentiation cascade (above) to determine the cell populations targeted by various antidepressant therapies in the adult and developing brain. We aim to determine whether different types of treatments that regulate mood converge on the same step in the cascade or if each has their own target populations, and to identify signaling molecules that translate the treatment into an increased number of new neurons. We have now determined the neurogenic targets of the SSRI (selective serotonin reuptake inhibitor) antidepressant fluoxetine, of electroconvulsive shock, of deep brain stimulation, of physical exercise, and of cosmic radiation. We are using the information we have developed to identify signals that control the quiescence and self-renewal of stem cells, which direct the differentiation cascade in the adult brain and which can be employed to repair the damaged and aging brain.

IDENTIFYING NEURAL PROGENITOR CELLS IN THE LIVE HUMAN BRAIN

To harness the full potential of stem cells in the brain, it is necessary to be able to identify and track those cells in the live brain. However, the present imaging technologies cannot be applied for identification of neural stem cells in the human brain. In a collaborative effort with Drs. Mirjana Maletic-Savatic, Petar Djuric, Helene Benveniste, and Fritz Henn from Stony Brook University and Brookhaven National Laboratory, we used proton magnetic resonance spectroscopy (^{1}H-MRS) to develop a new approach for detecting stem and progenitor cells in the live brain.

We started by identifying and characterizing a new biomarker enriched in neural stem and progenitor cells and demonstrated its use as a reference for monitoring neurogenesis in vitro. To detect low concentration of stem/progenitor cells in vivo, a signal processing method was developed that enabled the use of ^{1}H-MRS for the analysis of the biomarker in both rodent brain and the hippocampus of live human subjects. We found that the biomarker can be detected in the hippocampus but not the cortex of adult animals and humans, that it can be seen in stem cells transplanted into the cortex of recipient animals, and that its relative concentration increases when animals are subjected to electroconvulsive shock, which is a potent inducer of hippocampal neurogenesis. Finally, we found that the biomarker is higher in younger, as compared to older, tested patients, consistent with studies, including those from our group, that hippocampal neurogenesis strongly decreases with age. Our findings open the possibility of investigating the role of stem cells and neurogenesis in a wide variety of human brain disorders, including psychiatric and neurological disorders, and of evaluating the efficiency of therapeutic interventions.

STEM CELLS IN NONNEURAL TISSUES

Tissue maintenance requires a constant supply of new cells to replace those lost to stress and damage or destroyed as part of a normal cell turnover program. This replacement is made possible either by self-renewal of differentiated cells or through the activity of tissue-specific stem cells. Adult stem cells are usually morphologically unspecialized, can survive for a long time or undergo long-term self-renewal, and are located in specialized niches that restrict their division and support their undifferentiated status.

We found that in our reporter mouse lines, originally designed to mark neural stem cells, the nestin-driven reporter expression also marks stem and progenitor cells in a range of nonneuronal tissues. Expression of the reporter can be seen in stem and progenitor cell populations of the hair follicle, liver, pancreas, skeletal muscle, testis, and anterior pituitary. Furthermore, in several other organs and tissues, the nestin-driven reporter is expressed in locations expected for stem/progenitor cells of those tissues (e.g., prostate, lungs, ovaries, thyroid, spinal cord, kidney, and mammary grand). These findings suggest a close relation between the expression of Nestin and the stem-like properties of cells in the adult tissues. Furthermore, they provide a means to isolate stem and progenitor cells from those tissues, to use genetic inducible fate mapping to identify and isolate the progeny of such cells, and to examine the mecha-

nisms that govern quiescence, division, differentiation, death, plasticity, and self-renewal of stem cells in the adult tissues.

NO, STEM CELLS, AND DEVELOPMENT

Nitric oxide (NO), a crucial regulator of vasodilation, immunity, and neurotransmission, is also involved in regulating the balance between proliferation and differentiation in several developmental and differentiation settings. NO can act both in autocrine and paracrine signaling modes, activating soluble guanylate cyclase, combining with reactive oxygen species, modifying proteins, and inducing both short-term and long-term signaling cascades.

We are interested in the mechanisms of NO signaling and in its role in development and stem cell maintenance. Among our recent advances are discoveries of a new gene that mediates the action of NO, of the role that NO has in regulating hematopoiesis, and of its role in nervous system development.

We found a new NO- and stress-induced gene that we named *noxin*, and we generated a knockout mouse line where the *noxin* gene was inactivated. *noxin* expression is under tight control by the cell cycle but can be strongly induced by a range of stress signals. *noxin* expression arrests the cell cycle at G_1 in a p53-independent fashion. Importantly, loss or down-regulation of *noxin* expression leads to increased cell death. Our results suggest that *noxin* is a component of the cell defense system: It is activated by various stress stimuli, helps cells to withdraw from cycling, and opposes apoptosis. We are now analyzing *noxin* mutant mice to determine the function of *noxin* in organism development and in response to stress.

We further found that the neuronal NO synthase isoform (nNOS) regulates hematopoiesis in vitro and in vivo. nNOS is expressed in adult bone marrow and fetal liver and is enriched in stromal cells, and its inactivation increases the number of hematopoietic progenitors in the bone marrow and spleen. Our results describe a new role for nNOS beyond its action in the brain and muscle and suggest a model where nNOS, expressed in stromal cells, produces NO which acts as a paracrine regulator of hematopoietic stem cells.

Finally, we have found that during development, NO coordinates two major morphogenetic processes, cell division and cell movement. NO suppresses cell division and facilitates cell movement, such that inhibition of NO synthase during early development of *Xenopus* increases proliferation in the neuroectoderm and suppresses convergent extension in the axial mesoderm and neuroectoderm. NO controls cell division and cell movement through two separate signaling pathways (Fig. 2). Both rely on RhoA-ROCK signaling but can be distinguished by the involvement of either guanylate cyclase or the planar cell polarity regulator Dishevelled. Through the cGMP-dependent pathway, NO suppresses cell division by negatively regulating RhoA and controlling the nuclear distribution of ROCK and p21WAF1. Through the cGMP-independent pathway, NO facilitates cell movement by regulating the intracellular distribution and level of Dishevelled and the activity of RhoA, thereby controlling the activity of ROCK and regulating actin cytoskeleton remodeling and cell polarization. Concurrent control by NO helps ensure that the crucial processes of cell proliferation and morphogenetic movements are coordinated during early development. This study demonstrates the function of NO in planar cell polarity and highlights its role as a morphogenetic regulator.

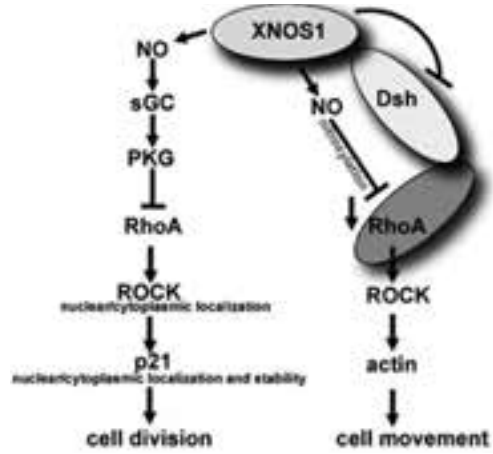

FIGURE 2. Schematic representation of the signaling pathways mediating the action of XNOS1 during *Xenopus* development (Peunova et al. 2007).

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Juan Encinas

MOLECULAR ANALYSIS OF NEURONAL RECEPTORS AND ION CHANNELS

H. Furukawa E. Karakas
N. Simorowski

Research in my group aims to understand the molecular basis for biological activity and physical properties of integral membrane receptors and ion channels involved in neurotransmission. To achieve our goals, three-dimensional structures are determined primarily by X-ray crystallography, and the functional properties of target proteins are assessed by structure-based site-directed mutagenesis in combination with biophysical and biochemical techniques including electrophysiology.

Specifically, we are focusing on three classes of integral membrane proteins, which demonstrate key roles in mediating neuronal activities: *N*-methyl-D-aspartate receptors (NMDARs), a ligand-gated cation channel that opens upon binding to glutamate and glycine and mediates excitatory synaptic transmission; low-density lipoprotein (LDL) receptor-related protein (LRP), a lipoprotein receptor that associates with NMDARs and regulates the strength of synaptic transmission; and γ-secretase, an intramembrane cleaving protease that mediates regulated intramembrane proteolysis of amyloid precursor proteins and LRP. The functions of the three classes of integral membrane proteins above are interrelated. Dysfunction of these proteins is implicated in various neurological disorders and diseases including Alzheimer's disease, Parkinson's disease, seizure, strokes, and schizophrenia. Our long-term goal is to understand the molecular mechanism for functional alteration of these proteins in disease states.

MECHANISM OF SUBTYPE SPECIFICITY IN NMDA-Rs

In 2007, we spent the majority of our effort in characterizing the mechanism of ligand binding on NMDARs by conducting X-ray crystallographic studies on the ligand-binding domain of the NR2D subunit (NR2D S1S2). The NMDAR ion channels containing NR2D have distinct properties characterized by exceptionally longer deactivation kinetics and a higher affinity for glutamate compared to those containing other NR2 subtypes (NR2A-C). Because each NMDAR subunit has distinct physiological roles, a subunit specific compound is preferred for the design of potential drugs. The atomic information of the NR2D S1S2 may prove useful for such purpose.

The bottleneck in X-ray crystallography is the formation of high-quality crystals that diffract X-ray at a level suitable for structural analysis. To acquire such crystals, a sufficient (milligram order) amount of the target proteins must be produced and purified to homo-

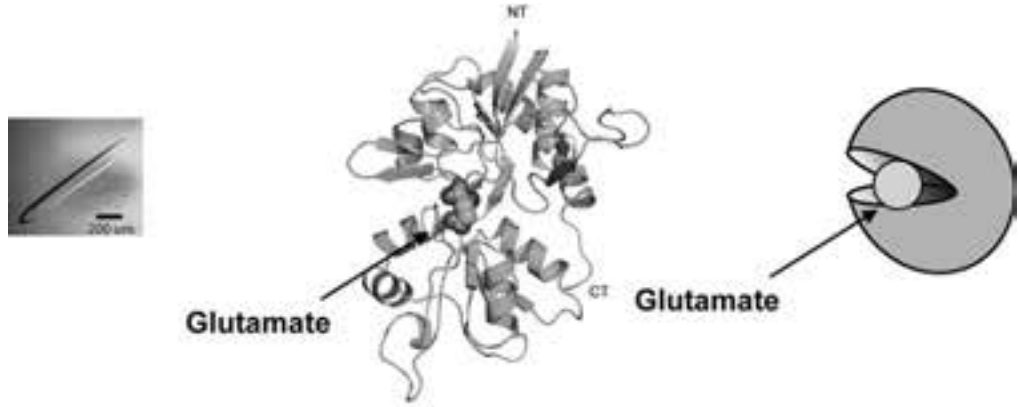

FIGURE 1 Typical morphology of the NR2D S1S2 glutamate crystals with X-ray diffraction at 1.7 Å. Shown in the middle is the "clam shell" structure of NR2D S1S2 in complex with glutamate (*sphere*). Glutamate is bound to the interdomain cleft between the upper and lower domain of the "clam shell."

geneity. Studies on NR2D have lagged behind due to difficulties in acquiring such protein samples. We have conducted factorial screenings on the expression of NR2D S1S2 and found conditions that allow us to obtain 4 mg of the NR2D S1S2 proteins from 20 liters of bacterial culture. The NR2D S1S2 proteins were purified to homogeneity by the four-step purification method involving Ni-NTA, anion- and cation-exchange chromatography, and size-exclusion chromatography. By using homogeneously purified proteins, we were able to find conditions to produce crystals that diffracted to 1.7-Å resolution. The X-ray diffraction data were collected at the National Synchrotron Light Source at Brookhaven National Laboratory. Using the X-ray diffraction data, we recently obtained atomic structures of the NR2D S1S2 ligand-binding core bound to different ligands including glutamate, NMDA, and aspartate. We are currently scrutinizing atomic information of NR2D S1S2 to understand the molecular mechanism underlying slow deactivation and NR2D subunit specificity. Along the way, we are designing functional experiments to assess structure-based hypothesis by electrophysiological techniques.

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Erkan Karakas

CONSTRUCTION AND FUNCTION OF THE GABA INHIBITORY CIRCUITS

Z.J. Huang Y. Fu, M. He, S. Kuhlman, D. Kvitsiani, M. Lazarus, J. Lu, R. Paik, A. Paul, H. Taniguchi, P. Wu, X. Wu, H. Yang

A major challenge in neuroscience is to understand how behaviors emerge from the underlying neural networks and their cellular constituents. In many areas of the vertebrate brain, GABAergic interneurons are crucial in establishing the functional balance, complexity, and computational architecture of neural networks. GABA interneurons regulate synaptic integration, probability, and timing of action potential generation in glutamatergic principal neurons; they further generate and maintain network oscillations, which provide temporal structures for orchestrating activities of neuronal populations. The rich variety and fine details of inhibition are achieved by diverse types of interneurons, which display distinct morphology, physiological properties, and connectivity patterns. The physiological properties of interneurons are optimized for generating a rich array of firing patterns and synaptic dynamics to precisely and efficiently control electrical signaling in neurons and networks. Furthermore, different classes of interneurons display characteristic axon arbors and innervation patterns, which distribute their outputs to discrete spatial locations, cell types, and subcellular compartments in the network. Like glutamatergic connections, GABAergic synapses are also modified by usage, leading to reconfiguration of the inhibitory circuits by experience. Understanding the organization, operation, and plasticity of GABAergic circuits is key to discovering general principles that govern how information is processed by neural circuits. My laboratory combines genetic, imaging, and physiological approaches to study GABAergic interneurons and circuitry in the cerebral and cerebellar cortex.

MOLECULAR MECHANISMS UNDERLYING SUBCELLULAR ORGANIZATION OF GABAERGIC SYNAPSES

Large principal neurons, such as neocortical pyramidal neurons and cerebellar Purkinje neurons, consist of distinct anatomical and functional compartments; such distributed and compartmentalized processing greatly increases the computational power of single neurons. These physiological compartments arise from subcellular distribution of all major classes of ion channels, receptors, and signaling mechanisms. Superimposed upon these biophysical compartments is the subcellular organization of synaptic connectivity, especially GABA inhibitory synapses, which is essential for regulating integration, spike timing, and back propagation in principal neurons.

Until our discovery, the mechanisms underlying subcellular synapse organization were almost entirely unknown. We have demonstrated that subcellular targeting of GABAergic synapses is guided by genetically determined mechanisms and does not involve experience-dependent neural activity (Chattopadhyaya et al., *J. Neurosci. 24:* 9598–9611 [2004]). Furthermore, we discovered that the subcellular distribution of an L1 family immunoglobulin protein (neurofascin186), recruited by an ankyrin family membrane skeleton (ankyrinG480), is a key mechanism that directs basket interneurons (Fig. 1) to innervate the axon initial segment of Purkinje neurons (Ango et al., *Cell 119:* 257–272 [2004]). Because ankyrinG also recruits voltage-gated sodium channels to AIS (axon initial segment) to promote the initiation of action potentials, "subcellular alignment" of ion channels and a specific class of inhibitory input may optimize the regulation of spike generation and timing in principal neurons. Importantly, the L1CAM (L1 cell adhesion molecule) and ankyrin families consist of different members that localize to distinct subcellular compartments. We recently discovered that another member of L1CAMs, CHL1 (close homolog of L1), contributes to the innervation of Purkinje cell dendrites by stellate interneurons (F. Ango et al., in press). Our findings support the general hypothesis that L1CAMs recruited to subcellular domains by different ankyrins regulate subcellular organization of GABAergic synapses.

ACTIVITY-DEPENDENT MATURATION AND PLASTICITY OF INHIBITORY SYNAPSES AND CIRCUITS

In addition to genetic programs, the construction of neural circuits requires activity-dependent regulation of neurite growth and synapse formation. As key mediators of

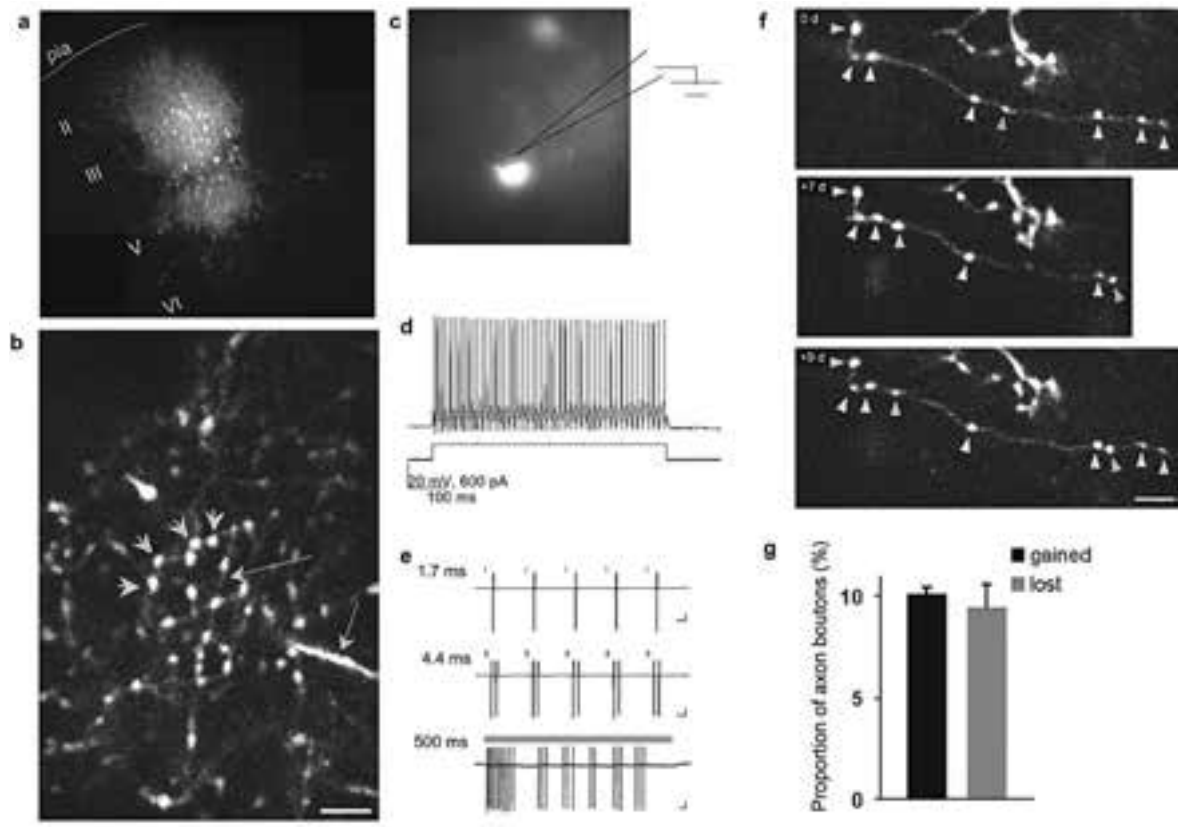

FIGURE 1 In vivo imaging of basket interneurons.

neural activity, neurotransmitters are particularly well suited to sculpt connections by coupling synaptic transmission with synaptic growth and refinement. Indeed, glutamate signaling regulates nearly all aspects of excitatory synapse development and plasticity. The development of inhibitory synapses and circuits is also regulated by neural activity, but the underlying mechanisms are poorly understood. In particular, it is unknown whether the inhibitory transmitter GABA itself plays a part. We have demonstrated that the maturation of inhibitory innervation targeted to the soma and proximal dendrites of pyramidal neurons in visual cortex proceeds into adolescence and is regulated by visual experience (Chattopadhyaya et al., *J. Neurosci. 24:* 9598–9611 [2004]).

We recently made a fundamental discovery that GABA and its rate-limiting synthetic enzyme GAD67 act beyond their classic roles in inhibitory transmission and mediate activity-dependent maturation and plasticity of inhibitory synapses and innervation patterns (Chattopadhyaya et al. 2007). We hypothesize that, similar to the role of glutamate in the development of excitatory synapses, GABA signaling is an activity-dependent mechanism to promote and coordinate presynaptic and postsynaptic maturation during the development of inhibitory synapses and axon arbors. Because GAD67 level is coupled to neuronal inputs, activity-dependent GABA synthesis and signaling may provide a cell-wide as well as synaptic mechanism to sculpt GABAergic connectivity patterns. Our discovery provides major insights into the fundamental mechanism of activity-dependent maturation and plasticity of inhibitory circuitry and raises a series of questions regarding the functional significance and underlying cellular and molecular mechanisms.

Maturation of GABAergic circuits in the developing visual cortex is regulated by sensory experience and, in turn, influences the critical period of ocular dominance (OD) plasticity. We discovered a role for polysialic acid (PSA), presented by the neural cell adhesion molecule (NCAM), in the maturation of GABA innervation and OD plasticity. Our results suggest that developmental and activity-dependent removal of PSA is a permissive

mechanism that regulates the timing of the maturation of GABA inhibition and onset of OD plasticity (di Cristo et al. 2007).

GENETIC TOOLS TO VISUALIZE AND MANIPULATE GABAERGIC NEURONS AND CIRCUITS IN VIVO

The diversity and complexity of GABAergic cell types are the basis for multifaceted functions of the GABA system but have been largely impenetrable by conventional anatomical and physiological techniques. Genetic strategies hold the promise to "deconvolute" this complexity because they tap into the intrinsic gene regulatory mechanism and logic that generate and maintain the GABAergic system. We are building genetic tools using Cre/loxP recombination-regulated gene expression to (1) visualize the morphology and connectivity of GABA interneurons with synapse resolution, (2) visualize the activity and activity history of interneurons, and (3) manipulate the firing and synaptic transmission of defined classes of cells at physiological time scales.

Using recombineering technology, we are generating more than 20 knockin "driver lines" expressing Cre or inducible CreER to establish "genetic access" to all major classes GABAergic neurons. In addition, we are also constructing a new generation of Cre-activated "reporter" mice to achieve high-level expression of fluorescent proteins (FPs) and "molecular switches"(MSs, such as ligand- or light-induced ion channels) in vivo, by incorporating a variety of amplification strategies. In collaboration with Sacha Nelson at Brandeis University, we are characterizing these drivers and reporter lines and establishing a Web-based platform for disseminating the mice, reagents, and expression data (UO1-MH078844-01; NIH Neuroscience Blueprint). These driver mice will significantly accelerate progress in studying all aspects of the GABAergic system.

Importantly, we recently incorporated virus-mediated gene delivery into our scheme: We developed a method that combines Cre-driver and Cre-activated adeno-associated virus (AAV) vectors to achieve high-level and cell-type-specific gene expression in the mouse brain. This method allowed us to image, for the first time, defined classes of inhibitory synapses in the live neocortex in vivo. The engineering of AAV vectors is simple and economic, and viral transfection can be directed to specific brain regions and developmental stages. We have now generated "conditional AAVs" expressing channnelrhodopsin, halorhodopsin, and allostatin to manipulate the activity of inhibitory neurons in a number of our projects. We are further developing this strategy to include viruses with larger capacity or *trans*-synaptic properties. The genetic access to specific cell types defined by Cre driver mice combined with efficient and flexible delivery of an array of conditional viruses to visualize and manipulate these cell types will herald a new era for studying the organization and function of complex neural circuits.

GENE EXPRESSION PROFILE AND PROGRAMS IN DIFFERENT CLASSES OF GABAERGIC INTERNEURONS

The stunning heterogeneity and complexity of GABAergic interneurons in their intrinsic and synaptic physiology, morphology, and connectivity are likely conferred by differential gene expression. A systematic characterization of gene expression profiles among GABAergic cell types may provide the most comprehensive and quantitative description of their molecular make-up and yield fundamental insight into their classification, biophysical repertoire, and the genetic program directing their development. We have generated an array of bacterial artificial chromosome (BAC) transgenic mice expressing green fluorescent protein (GFP) in different classes of GABAergic neurons. In collaboration with my laboratory, Sacha Nelson at Brandeis University has developed a method to manually purify GABA neurons for microarray analysis (Sugino et al., *Nat. Neurosci. 9:* 99 [2006]). We are applying this method to the development of the neural circuit in the cerebellar cortex.

The cerebellum is appealing because various transgenic mice label every major class of GABAergic neurons and glia cells throughout development. These studies may reveal novel biophysical properties, signaling pathways, and transcription programs among interneurons and also yield better tools (such as genes that define novel cell types) for more refined genetic manipulations. Such a non-hypothesis-driven approach is essential to elucidate the genetic design of the GABAergic system and its function and capacity for plasticity.

GABAERGIC CIRCUITS IN NEURODEVELOPMENTAL DISORDERS

We have recently received major funding from the Simons Autism Research Initiative to study Rett Syndrome (RTT), the best characterized form of the autism spectrum disorder, 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.

In collaboration of Dr. Sacha Nelson at Brandeis University, we will combine genetic, genomic, imaging, and physiological approaches to (1) identify the subset of GABAergic neurons in specific brain regions that are influenced by *MeCP2* mutations at defined developmental stages and during experience-induced plasticity, (2) characterize the impact of *MeCP2* mutations on gene expression profiles in specific classes of neocortical interneurons, and (3) determine the impact of *MeCP2* mutations on the physiological properties and synaptic innervation pattern of specific classes of neocortical interneurons.

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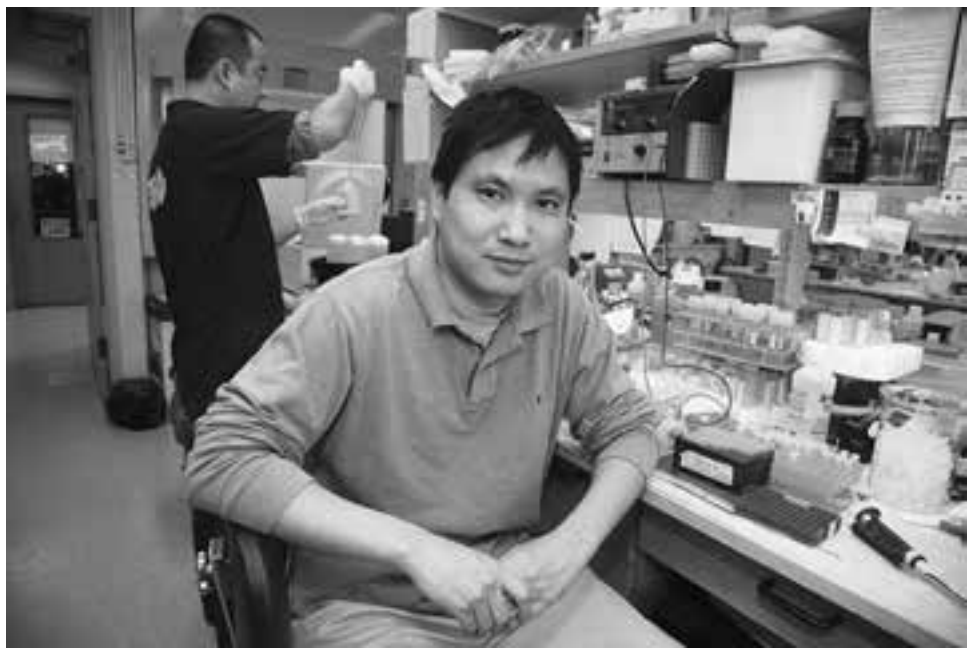

Rae Hun Paik and Hong Yang

NEURAL CIRCUIT DYNAMICS UNDERLYING DECISION MAKING

A. Kepecs D. Kvitsiani

Research in my newly established laboratory focuses on the computational principles and neurobiological mechanisms of decision making. We view complex behaviors as sequences of elementary decisions, which can be conveniently studied in isolation to expose their underlying neural processes. Yet, even simple decisions involve the integration of sensory and memory information with emotional and motivational attributes requiring the concerted action of millions of neurons across brain regions. Therefore, we take an integrative approach, combining tightly controlled rodent behavior with electrophysiological recordings to monitor neural activity, molecular tools to perturb genetically identified neural circuit elements, and quantitative analysis to guide and sharpen the neurobiological questions.

At present, we are pursuing two broad directions. First, we seek to identify new neurocomputational principles that go beyond the sensory and motor processes of decision making by studying more elusive attributes such as emotion, motivation, or confidence. Recently, we discovered neural correlates of decision confidence in the orbitofrontal cortex of rats, and we are currently investigating both the neural mechanisms and algorithms by which this signal supports adaptive behavior. Second, we want to understand how specific cell types participate in the neural circuit dynamics of local processing, and how different brain regions with specialized functions coordinate their activity. Toward this goal, we are adapting molecular and optical tools for use in behaving animals to identify and manipulate defined neuronal subtypes and pathways.

Ultimately, we would like to gain mechanistic insights into decision processes in the hope that these will be of utility not only for a basic scientific understanding, but also to lead to better treatments for brain dysfunctions of decision making, such as pathological gambling, drug abuse, and anxiety disorders.

Role of Orbitofrontal Cortex in Evaluating Decision Confidence

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

If you are asked to evaluate your confidence in something you know—how sure are you that it is true—you can readily answer. What is the neural basis for such judgments? Is knowledge about beliefs an example of the human brain's capacity for self-awareness? Or is there a simpler explanation that might suggest a more basic yet fundamental role for uncertainty in neural computation?

In collaboration with Naoshige Uchida (Harvard University), Hatim Zariwala (CSHL), and Zach Mainen (CSHL), we addressed these questions by experimentally manipulating the difficulty of perceptual decisions and searched for a neural correlate of rats' confidence about getting a reward. We trained rats in a two-choice odor-mixture categorization task that is well suited for studying decision making in rodents. In this task, rats report the dominant odor-mixture component by entering different choice ports. Their performance is nearly perfect for pure odors and drops systematically with mixture ratio (difficulty). We found a population of neurons in orbitofrontal cortex (OFC) whose firing tracked the difficulty of the decision and, surprisingly, could even anticipate the outcomes of different trials of the same difficulty. We were able to concisely explain this pattern of data using a simple model of decision confidence. We then devised a behavior, which showed that rats could actually report their confidence when probed with a subsequent decision. This demonstrated that rats, like us, sometimes know how certain they are in their answers. Taken together, our results support the idea that decision confidence may be a widespread and fundamental element of neural processing contributing to adaptive behavior.

We seek to extend these initial observations by trying to establish that confidence-related neural activity in the OFC is *causally related* to confidence judgments. This key issue will be approached several ways. First, we will refine a variant of our categorization task where the optimal amount of time to wait for a reward depends quantitatively on the confidence in a decision. We will then be in a position to ask whether confidence-related neural activity in OFC can predict the timing of leaving decisions on a trial-by-trial basis. Such result would support the hypothesis that OFC contributes directly to an uncertainty-guided behavior. Even if successful, it remains possible that similar representations exist elsewhere in the brain. Therefore, we will test whether OFC

has a primary role in confidence-guided behaviors by inactivating it. We hypothesize that shutting off OFC leaves the odor-guided behavior intact while impairing the use of confidence information to guide leaving decisions. In collaboration with Erin Romberg (Oberlin) and Zach Mainen (CSHL), we are testing this hypothesis. Our preliminary results show that lesions of OFC significantly impair rats' ability to report confidence by waiting for delayed rewards without affecting olfactory discrimination performance. Notably, these findings are consistent with studies of human patients with orbitofrontal lesions who are unable to judge the degree of uncertainty, indicating that the functions of OFC we are investigating in rats are conserved across species.

These results suggest that a dynamic representation of and access to an internal measure of decision confidence is fundamental to adaptive behavior—even in rats—and provide insights into the nature of the neural processes underlying decision making. Our findings establish neuronal correlates and behavioral assays that lay a strong foundation for a broader examination of the neural mechanisms underlying confidence estimation.

Gambling Rats: A New Behavioral Task to Study Confidence Judgments

A. Kepecs [in collaboration with L.-H. Tai and A. Zador, Cold Spring Harbor Laboratory]

As humans, we assess and cope with uncertainty all the time. Knowing how uncertain we are confers significant benefits for a broad range of activities from the sophisticated—managing a stock portfolio—to the mundane—how much to bet in poker. Our previous studies established that rats can report their confidence by the length of time they are willing to wait for delayed rewards. Here, we seek to establish another behavioral task where rats will explicitly report their confidence by entering different response ports.

Toward this goal, we are training rats in an auditory tone categorization task (Fig. 1). Rats can initiate trials by entering the center port that triggers the delivery of an auditory tone. They are trained to respond to low tones on the left and high tones on the right to receive water rewards for correct choices. By varying the frequency of tones, we can systematically change the difficulty of decisions: Tones near the category boundary result in low accuracy, whereas tones far away from the boundary produce high accuracy. In the "gambling" version of this task, rats get an additional choice on each response side between a near or "safe" port and a far or "risky" port (Fig. 1). For correct responses, the "safe" ports yield only a small amount of water reward compared to "risky" ports. On the other hand, incorrect responses to "risky" ports produce long time-out periods, during which new trials cannot be initiated. Thus, the optimal strategy for the rat is to "bet risky" when certain and receive a large water reward and "bet safe" when uncertain to avoid the long time-out period for incorrect choices. We plan to examine whether rats are able to act on their degree of confidence and use this optimal "gambling" strategy.

It has been argued that acting on the degree of confidence requires sophisticated metacognitive capacity requiring awareness and a neural architecture specific to humans and other primates. Interestingly, there is a rare human condition called blindsight in people with injuries to early visual cortex, so that when patients are asked to make a visual choice, they deny the awareness of visual stimuli—and insist that they are guessing—yet perform well above chance level. On the other hand, because they do not seem to be aware of visual stimuli, they cannot make appropriate confidence judgments. Consequently, using a gambling paradigm, it has been shown that people with blindsight wager money based on their awareness, rather than performance, suggesting

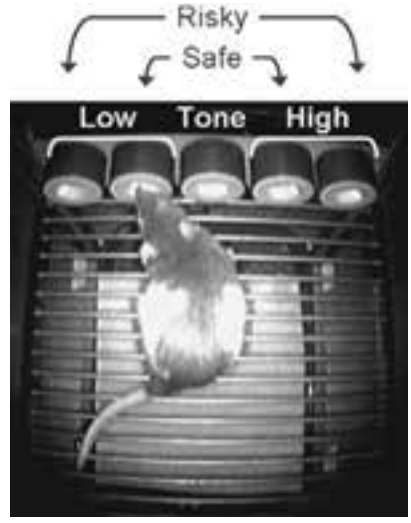

FIGURE 1 Rat gambling task. A rat performing an auditory tone categorization task. For low tones, the correct answer is left, and for high tones, it is right. For each side, the rat has two options, the near "safe" ports or the far "risky" ports. For correct responses, the "safe" ports yield only a small amount of water reward compared to "risky" ports, but an incorrect response to "risky" ports results in a time-out punishment.

that wagers can provide a way to quantitatively measure awareness. Therefore, by establishing a behavioral paradigm to explicitly measure decision confidence on a trial-by-trial basis, we will be in a position to ask mechanistic questions about the neural processes underlying auditory awareness.

How Uncertainty Boosts Learning: Dynamic Updating of Decision Strategies

A. Kepecs [in collaboration with N. Uchida, Harvard; Z. Mainen, Cold Spring Harbor Laboratory]

Making optimal decisions requires that decision strategies be updated based on the successes and failures of similar decisions made in the past. Specifically, reinforcement learning theory proposes that a quantitative prediction error—the difference between expected and observed outcomes—drives learning. Moreover, according to statistical learning theory, the size of the updates, i.e., the degree of learning, should depend on the current estimates of uncertainty: Learn more when uncertain and less when certain. However, there is only limited direct evidence for these predictions, in part because quantitative measurements of learning increments are difficult to assay experimentally.

If such learning processes are active, they may provide ongoing behavioral adjustments even in the absence of explicit changes in behavioral contingencies that would require overt learning. For example, during categorization tasks, the decision boundary can be learned through reinforcement, but it may undergo continual updating during performance thereafter. Therefore, we used an olfactory categorization task to examine the trial-by-trial updating of behavioral strategy.

Interestingly, we found that animals were dynamically adjusting their decision strategy even after extensive training. For difficult decisions (those near the category boundary), the outcome is very informative about location of the decision boundary, whereas the outcome of pure odor trials (far from the decision boundary) reveals little about the boundary. Accordingly, the decision boundary should be adjusted more following difficult trials with high uncertainty than for trials with no uncertainty. Indeed, we found that rats biased their decisions toward the more recently rewarded direction as if their decision boundary was shifted. Moreover, the magnitude of this bias was proportional to the uncertainty of the previous decision, as predicted. This bias, however, was only observed for difficult decisions, suggesting that the category boundary and not the side-bias was being updated. Thus, rats not only have an estimate of their decision uncertainty, but this estimate also appears to contribute to future decisions. We are currently testing simple reinforcement models to quantitatively account for these results. Our observations of dynamic changes in decision strategies even during well-learned behaviors suggest that a theoretically optimal updating strategy based on graded prediction error signals applies generally across species and different learning paradigms.

Neural Circuit Dynamics of Genetically Identified Neurons in Behaving Mice

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

In vitro studies of neural circuits provide a picture of the brain with exquisite precision in neural connections and dynamics between specific neural cell types. In contrast, systems neuroscience studies revealed how the activity of single neurons is correlated with perception and behavior, albeit without knowing the identity of the recorded neurons. Our goal is to bridge this gap by developing a method to determine the molecular identity of the neurons recorded during behavior.

The availability of genetically targetable fluorescent markers and activity reporters has revolutionized cellular neuroscience, and similarly, the recently characterized ChannelRhodopsin-2 (ChR2), a light-sensitive cation channel, promises to do the same for systems neuroscience. Using ChR2, genetically defined neuron classes can be made light-activatable so that we can optically control the generation of spikes with millisecond precision. We will use this optical control to identify neurons using traditional extracellular electrodes that can be used in behaving animals. Briefly, we will introduce fiberoptic probes to deliver light along with our electrodes. In this configuration, genetically specific neuron populations expressing ChR2 can be unambiguously separated on a recording electrode from the rest of the neuronal population using light stimulation.

We will use this tool to understand how the diversity of hippocampal inhibitory neuron cell types contributes to neural circuit dynamics. Specifically, it is known that the hippocampus exhibits different states of activity and this network activity is organized as oscillations spanning multiple timescales. The circuit dynamics is known to change depending on a behav-

ioral state of an animal. For instance, hippocampal oscillations switch between distinct frequency bands across different sleep states or when an animal enters into active exploration mode. How are these distinct modes of network dynamics generated and controlled? What are the roles of different neural populations in circuit dynamics? We hope to answer these questions by adapting these optical-genetic tools for use in behaving mice.

Toward this goal, we are collaborating with Josh Huang's lab here at CSHL to generate knockin mouse lines labeling different populations of inhibitory interneurons with Cre recombinase. Along with the Cre lines, we are generating a reporter mouse line carrying ChR2-coding sequences under a strong ubiquitous promoter interspersed by a floxed transcriptional stop cassette. These reporter mice will be bred to Cre mouse lines to activate ChR2 in distinct interneuron classes. As an alternative to this approach, we have managed to use a viral strategy to conditionally express the very same proteins in mouse brain. Specifically, we used adeno-associated virurses (AAV) to deliver Cre activatable ChR2 protein into the hippocampus. This approach achieved a high level of protein expression that appears sufficient to be able to activate these neurons with light. Our next step will be to develop custom-designed "drives" that can be used to implant different fiber optic probes in combination with tetrodes (four-wire electrodes). This will also require miniaturizing our current "drives" so that they can be implanted in freely behaving mice. Ultimately, we plan to use this strategy to record and at the same time identify specific neural classes in behaving animals in other brain areas and behaviors as well.

The development of these tools will also allow us to test the roles of different neural classes by directly stimulating them and observing the consequences on global neural activity and behavior. This project is an example of a new era in systems neuroscience, where molecularly identified circuit elements can be monitored and disrupted at high temporal precision.

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BIOPHYSICAL BASIS FOR NEURAL COMPUTATION

A. Koulakov D. Tsigankov

Our laboratory studies theoretical models for the development of connectivity in the brain. We attempt to understand how the effects of binding and activation of chemical labels, such as Eph receptors and their ligands, ephrins, can be combined with Hebbian plasticity. General methods advanced by us this year represent formation of more abstract neural network as a result of interplay between genetic factors represented by molecular labels and environmental information given by the neuronal electric activity. Our theoretical methods allow us to study the dynamics and configurations of biologically realistic neural networks.

Sperry versus Hebb: Topographic Mapping in Isl2/EphA3 Mutant Mice

D. Tsigankov, A. Koulakov

In wild-type mice, axons of retinal ganglion cells establish topographically precise projection to the superior colliculus of the midbrain. This implies that axons of neighboring retinal ganglion cells project to the proximal locations in the target. The precision of topographic projection is a result of combined effects of molecular labels, such as Eph receptors and ephrins, and correlated electric activity. In Isl2/EphA3 mutant mice, the expression levels of molecular labels are changed. As a result, the topographic projection is rewired so that the neighborhood relationships between retinal cell axons are disrupted.

We argue that the effects of correlated activity presenting themselves in the form of Hebbian learning rules can facilitate the restoration of the topographic connectivity even when the molecular labels carry conflicting instructions (Tsigankov and Koulakov 2008). This occurs because the correlations in electric activity carry information about retinal cells' spatial location that is independent of molecular labels. We therefore argue that experiments in Isl2/EphA3 knock-in mice directly test the interaction between the effects of molecular labels and correlated activity during the development of neural connectivity (Fig. 1).

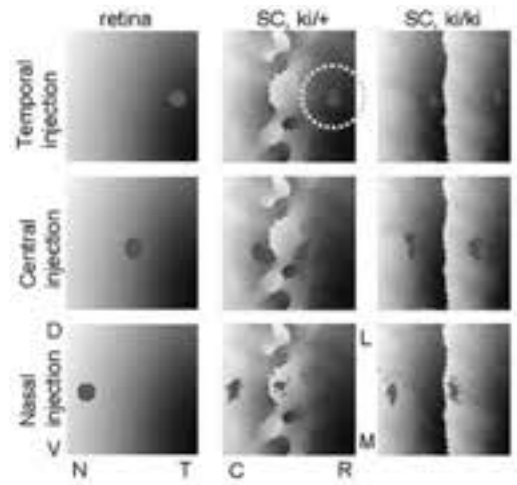

FIGURE 1 Topographic maps in Isl2/EphA3 knock-in mice as obtained by the computational model. The complete map structure is coded by shades of gray according to the axonal origin in retina as shown by the left column. In addition, the termination zones for a small subset of axons indicated by the points are shown for temporal (*upper row*), central (*center row*), and nasal (*lower row*) labeling. In mutant animals, the axons are split into two groups that terminate at different positions in the target. Thus, modifications of the molecular labels lead to the rewiring of brain connectivity. However, in the case of temporal injection in heterozygous knock-ins (*center, top*), two groups of axons blend into the same terminations zone (*dashed circle*). We argue here that this collapse of the two groups of axons occurs because of the effects of correlated neural activity, which are capable of fixing the map despite the counteracting molecular labels.

Optimal Axonal and Dendritic Branching Strategies during Development of Neural Circuitry

D. Tsigankov, A. Koulakov

In the developing brain, axons and dendrites are capable of connecting to each other with high precision. Recent advances in imaging allowed monitoring of the axon, dendrite, and synapse dynamics in vivo. It was observed that the majority of axon and dendrite branches formed are retracted later during the development. In this study, we computationally analyzed different axonal and dendritic branching strategies that minimize the number of

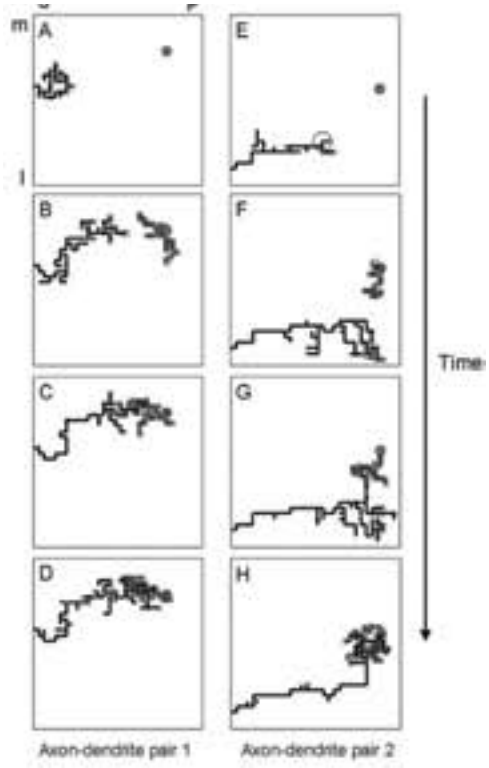

FIGURE 2 In silico structure of developing axons and dendrites. In the model axons and dendrites evolve in time by creating and eliminating new branches and retracting and elongating existing ones. The synapses (*circles*) are created and eliminated between the overlapping branch segments of axons and dendrites. Of the 900 simultaneously evolving axonal arbors, two are shown at sequential time frames (*A–D* and *E–H*). Two shown dendrites are the main recipients of the synaptic connections for two shown axons when topography is established. The axonal branching is biased toward their correct termination zone, providing the spatial overlap with the arbors of the appropriate dendrites. The synapses are then formed and maintained between appropriate axons and dendrites, thus forming a topographic mapping.

transient branches required to establish connectivity with particular precision. We applied these branching rules to the development of retinotectal topographic connectivity and found that axons and dendrites have different optimal branching strategies.

The axonal optimal strategy is to form new branches in the vicinity of existing synapses, whereas the optimal rule for dendrites is to form new branches preferentially in the vicinity of synapses with correlated presynaptic and postsynaptic electric activity. We show that experimentally observed different reactions to the NMDA (*N*-methyl-D-aspartate) receptor block in the dynamics of axonal and dendritic branching imply that that these two branching strategies are implemented in the developing brain. We suggest that the difference in branching strategies of axons and dendrites could be detected by measuring the spatial correlations between synapses and branch points on the developing arbors. We thus predict that these correlations should be reduced for dendrites but not for axons under the conditions of the NMDA receptor block.

Combinatorial ON/OFF Model for Olfactory Coding

A. Koulakov [in collaboration with D. Rinberg and A. Gelperin, Monell Chemical Senses Center, Philadelphia, Pennsylvania]

We study a model for olfactory coding based on spatial representation of glomerular responses. In this model, distinct odorants activate specific subsets of glomeruli, dependent on the odorant's concentration. The glomerular response specificities are understood statistically, based on experimentally measured distributions of detection thresholds. A simple version of the model, in which glomerular responses are binary (the on/off model), allows us to quantitatively account for the following results of human/rodent psychophysics: (1) Just-noticeable differences in the perceived concentration of a single odor (Weber ratios) are dC/C ~0.1; (2) the number of simultaneously perceived odors can be as high as 12; and (3) extensive lesions of the olfactory bulb do not lead to significant changes in detection/discrimination thresholds. We conclude that a combinatorial code based on a binary glomerular response is sufficient to account for the discrimination capacity of the mammalian olfactory system (Koulakov et al. 2007).

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THE NEURAL ORGANIZATION OF OLFACTORY BEHAVIOR

Z.F. Mainen C. Feierstein, G. Felsen, A. Kepecs, M. Murakami, M. Quirk, S. Ranade, M. Smear, D. Sosluski, M. Uchida, N. Xu, H.A. Zariwala

Our lab is studying the neural mechanisms of goal-directed behavior. We work with a simple but flexible psychophysical paradigm in which rats use odors to guide spatial choices to obtain reward. We are particularly interested in how olfactory information is encoded and transformed into adaptive decisions. We discovered several years ago that rats can respond accurately to sensory information extremely quickly—in a single sniff—and we are continuing to study the temporal limits and mechanisms of rapid olfactory discrimination as well as more complex computational problems such as concentration- and background-invariant odor recognition and odor-guided navigation.

To get at the underlying neural mechanisms, we are using chronic multielectrode recording to monitor single neuron activity and neural circuit interactions. We recently found that the orbitofrontal cortex integrates spatial and reward information to construct maps of behavioral goals. Ongoing recordings focus on temporal coding in the olfactory cortex and the dynamics of representations during learning. From a broader neuroeconomic perspective, we are also studying how the brain deals with the costs of decisions (effort, delay, uncertainty).

Finally, we are investigating how psychoactive drugs alter behavior, beginning with ketamine, a drug thought to mimic aspects of psychosis. We hypothesize that drug states can be understood as specific perturbations of neural circuit dynamics. An understanding of neuropharmacology at the circuit level may lead to more effective treatments for disorders such as Parkinson's disease and schizophrenia.

Should I Stay or Should I Go? Neural Mechanisms of Patience

M. Murakami

Consider the situation in which you wait for a bus but it is delayed. You have no idea when it arrives. There is another option, taking a cab, which costs much more but is available immediately. How long do you wait for the bus? When do you give up waiting for the bus and decide to take a cab? In such circumstances, your brain must compute the costs and benefits of the choices and use that information for choosing an action—waiting or giving up. In this study, we investigated a role of medial prefrontal cortex (mPFC) and secondary motor cortex (M2) in deciding to wait or not to wait.

We developed a task in which a rat is faced with a waiting problem similar to the "bus-or-cab" dilemma. There are two nose poke ports: One is for waiting and the other is for delivering a water reward. A short delay (400 msec) after a rat pokes in the waiting port, a first tone is played, and this signals the availability of a small reward at the reward port. If the rat goes to the reward port after the first tone, it receives a small amount of water reward. If the rat waits longer in the waiting port, instead of going to the reward port, then a second tone is played after a random delay period of up to several seconds. If the rat goes to the reward port after this second tone, it receives a larger amount of water reward. In this task, after the first tone is played, the rat is confronted with a choice between going to the reward port to get small reward and waiting for a second tone to get large reward.

Behavioral results showed that the time a rat is willing to wait for a given set of reward amounts and delays varies randomly from trial to trial. On some trials, the rat will respond immediately after the first tone; on other trials, it will withhold responding until after the second tone; and on others, it will wait well past the first tone but give up before the second.

We recorded neuronal activity from the mPFC and M2 during this task. We found that the activity of a subset of mPFC/M2 neurons can predict how long a rat will withhold a response, i.e., the firing rate of these neurons during a time window before the end of waiting was significantly correlated with waiting time of the rat. Individual neurons showed both positive and negative correlations with waiting time. Predictive activity occurred at different time points during the waiting period and even before the waiting period. These results support a possible role of mPFC/M2 in decision making of waiting or not waiting.

Movement Selection and Initiation in the Rat Superior Cilliculus

G. Felsen

We are interested in how the nervous system uses sensory stimuli to select and initiate appropriate motor actions. To this end, we have been studying how rats use olfactory cues to guide choices about, and to initiate, directional movements. We hypothesized that the superior colliculus (SC) could have an important role in these processes, since it is known to produce orienting movements, via descending projections to several motor nuclei. In rats, it is not known how activity in the SC underlies movement initiation and whether the SC is also involved in selecting among potential movements.

To address these questions, we used tetrodes to record from intermediate and deep-layer SC neurons in well-trained rats performing a two-alternative choice olfactory discrimination task. In each trial of the task, the rat first entered a centrally located odor port, triggering the delivery of an odor, and then moved to either the left or right reward port to obtain a water reward. The odor was either a pure compound or a mixture of two compounds. One pure odor was rewarded following a left choice and the other following a right choice, and mixtures were rewarded according to their dominant component.

We first analyzed activity during movement, as the rat moved both toward the reward port and back to the odor port. We expected to identify neurons that were selective for the direction of movement, consistent with previous results in primates. Although a population of neurons was direction-selective, we found a large fraction of neurons that preferred movement in one direction when moving toward the water port but in the other direction when returning to the odor port. These neurons can be thought of as selective for a region of space within the behavioral environment (e.g., the left side of the box). Such spatial selectivity in the SC has not been reported previously in rodents or primates, and together with directionally, selective neurons could have an important role in coordinating laterally orienting movements.

We next analyzed the activity during the premovement epoch, defined as the 100 msec preceding the exit from the odor port. The activity of many neurons during this epoch depended on whether the upcoming movement was leftward or rightward and did not depend on the identity of the odor being presented. Across the population, higher firing rates preceded movement contralateral to the recording site. In some cells, this direction selectivity disappeared at the onset of movement, whereas in other cells, it persisted during the movement.

These data suggest that prospective direction selectivity in the SC is involved in the initiation of laterally orienting movements. To determine whether the observed selectivity causes, or is simply correlated with, movement initiation, we used the $GABA_A$ agonist muscimol to unilaterally inactivate the SC in rats performing the behavioral task. If SC output is necessary for initiating contralateral movements, we would expect inactivation to bias the rat toward ipsilateral movements. Indeed, we found that muscimol, but not saline, delivered to the left SC biased the rat toward leftward choices, and this bias was dosage-dependent. Thus, normal SC activity is necessary for initiating appropriate directional movements.

Finally, we performed anatomical experiments to determine whether the SC receives input directly from an olfactory cortical region. Using a retrograde tracer, we identified a projection from the lateral entorhinal cortex, which is known to process olfactory information. This pathway suggests the existence of a parsimonious circuit for acting on olfactory-cued decisions.

Diversity and Precision of Neural Activity in Serotonergic Brainstem Nuclei

S. Ranade

Serotonin is a neurotransmitter implicated in a diverse range of physiological functions and behaviors as well psychiatric disorders including depression and anxiety. Serotonin is released by neurons in a set of brainstem nuclei called the raphe nuclei. The raphe system is by far the most complex, divergent neuromodulatory system in the brain. Our current understanding of serotonin function has been gained mostly from pharmacology and lesion studies. Neuronal recordings from animals performing well-controlled behavioral tasks have greatly increased our knowledge of dopamine and norepinephrine function, but there have been few recordings from raphe neurons during behavioral tasks. We hypothesize that such studies will yield novel insights about raphe function, particularly at fast timescales.

In this project, we recorded from raphe nuclei in rats performing a two-choice alternative odor discrimination task (as described above). Rats were trained to associate individual odors with water reward at one of two choice ports. Correct responses were rewarded probabilistically after a variable delay. This paradigm allowed us to study

sensory, motor, and reward-related responses with high temporal precision by aligning to nose poke events.

After training, rats were chronically implanted with a six-tetrode recording drive. Tetrodes were targeted to the raphe using a guide cannula. Recordings were obtained from 54 neurons in seven rats over an average of four to eight sessions per rat. Recording locations were verified histologically following termination of experiment. Raphe neurons showed diverse firing properties with respect to waveform characteristics, firing rate, and sleep state modulation. By conventional criteria, 10% of neurons were wide-spiking putative serotonin neurons. Neuronal responses were analyzed with respect to four epochs: odor sampling, movement, reward anticipation, and reward consumption. Firing rates of >70% neurons were modulated during at least one behavioral epoch, with a large fraction tuned to multiple events. Many neurons responded to behavioral events within 100 msec and some were even more precisely time-locked (order 20 msec), showing a very strong (>40 sp/sec) phasic response, apparently to the click produced by water valve opening.

A wide range of event-tuning characteristics were observed in the recorded population. During odor sampling, approximately one third of the units showed a decrease in firing rate. A subset of neurons also showed odor-induced activation, which, in rare cases, was stimulus-selective. During the movement epoch, an equal proportion of neurons showed enhancement and suppression of firing. There were few instances of direction-selective tuning. A large proportion of neurons (~40%) were inhibited during reward anticipation. A small subset of neurons (10%) showed changes in firing rate around the time of the expected reward. Putative serotonin neurons showed no obvious association with a specific response profile.

These recordings demonstrate that raphe neurons are rapidly and precisely modulated by diverse behavioral events. This is in accord with the known diversity of serotonin function and the difficulty in accounting for it with a simple unified theory. Functional diversity of raphe responses likely reflects in part the diversity of intrinsic properties and synaptic connectivity of neurons within the nucleus. Classification of units into putative serotonin and nonserotonin neurons did not yield any obvious simplification of response diversity, highlighting the need for methods relating firing patterns to precise identification of neuronal cell types. Finally, the observed functional diversity of raphe neurons is consistent with the possibility that significant information processing may occur within the raphe itself. Future studies will focus on developing novel molecular genetic approaches to selectively label and record activity of populations of raphe neurons that are biochemically homogeneous and/or share similar connectivity patterns.

Contextual Influences on Olfactory Sensory Processing

N. Xu

Cognitive processes such as attention and expectation are thought to exert top-down influences on sensory information processing, leading to differential behavioral responses as well as neural representations for identical sensory stimuli under different stimulus or behavioral contexts. How do such processes impact the neural representation of odors in the primary olfactory cortex?

To examine this issue, we designed an odor discrimination task (as described above) with varying occurrence probability of different odor mixture pairs in different blocks of trials. The relative stimulus probability was hypothesized to modulate the subjects' expectation for the forthcoming stimulus while performing a difficult odor discrimination. We found that this manipulation indeed effectively modulated the subjects' behavior. For difficult discrimination problems, performance accuracy was significantly reduced when the probability of occurrence of a given stimulus was lowered from 40% to 10%. For easy discrimination problems, no significant decrease was seen. These results provide behavioral evidence that olfactory discrimination depends on stimulus statistics, which would effectively modulate the subjects' expectation for forthcoming stimuli.

We next performed chronic multielectrode recording from the piriform cortex to test whether such modulation of odor discrimination correlates with a modulation of stimulus representation in the primary sensory cortex. We found that neuronal responses were remarkably modulated by the manipulation of stimulus statistics. Analyses of these data are ongoing.

The "Where" Problem in Olfaction: Behavioral Strategies and Neural Mechanisms

M. Smear

Localizing the source of an odor often matters as much as identifying it: Distinguishing a meal from a predator is futile if you avoid the former or approach the latter. Although our understanding of odor identification, the

"what" problem of olfaction, has progressed considerably, the "where" problem of olfaction has received relatively little attention. We know that rats excel at odor-guided navigation, but we do not know what behavioral strategies and neural circuits they use.

A critical challenge to elucidating the neural mechanisms underlying odor tracking is reducing the behavior to a form suitable to psychophysical techniques. To this end, we have designed an "olfactorium" which is essentially a two-armed maze in which the rat has to navigate to the correct arm based on concentration cues. Using a photoionization detector, we can obtain a statistical characterization of odor concentration patterns with high spatial, temporal, and concentration resolution. In addition, we can precisely track the nose position of a freely moving rat at video-rate time resolution in real time. By combining odor concentration measurements with tracked trajectories, we hope to reconstruct the time-varying odor concentration pattern experienced by a navigating rat, and thereby reverse engineer its navigation strategies. In the future, we intend to exploit rodent neuroscience's growing arsenal of tools for electrophysiology and transgenesis in the context of olfacto-spatial psychophysics to understand the neural circuitry of odor source localization.

The Neural Representation and Impact of Decision Confidence

A. Kepecs, H. Zariwala, N. Uchida

If you are asked to evaluate your confidence in something you know—how sure are you that it is true—you can readily answer. What is the neural basis for such judgments? Is knowledge about beliefs an example of the human brain's capacity for self-awareness? Or is there a simpler explanation that might suggest a more basic yet fundamental role for uncertainty in neural computation?

We addressed these questions by experimentally manipulating the difficulty of perceptual decisions and searched for a neural correlate of rats' confidence about getting a reward. We trained rats in a two-choice odor-mixture categorization task that is well suited for studying decision making in rodents. In this task, rats report the dominant odor-mixture component by entering different choice ports. Their performance is nearly perfect for pure odors and drops systematically with mixture ratio (difficulty). Using this task, we could systematically manipulate the uncertainty of individual discriminations by varying odor-mixture ratio.

We found a population of neurons in the orbitofrontal cortex (OFC) whose firing tracked the difficulty of the decision and, surprisingly, could even anticipate the outcomes of different trials of the same difficulty. We were able to concisely explain this pattern of data using a simple model of decision confidence. We then devised a behavior, which showed that rats could actually report their confidence when probed with a subsequent decision. This demonstrated that rats, like us, sometimes know how certain they are in their answers. Taken together, our results support the idea that decision confidence may be a widespread and fundamental element of neural processing contributing to adaptive behavior.

We began to extend these observations by testing whether confidence-related neural activity in the OFC is causally related to confidence judgments. Specifically, we began to test whether the OFC has a primary role in confidence-guided behaviors by inactivating it. We hypothesize that shutting off the OFC leaves the odor-guided behavior intact while impairing the use of confidence information to guide leaving-decisions. In collaboration with Erin Romberg here at CSHL (2007 URP), we are testing this hypothesis. Our preliminary results suggest that lesions of OFC indeed significantly impair the rats' ability to report confidence by waiting for delayed rewards without affecting olfactory discrimination performance. Notably, these findings are consistent with studies of human patients with orbitofrontal lesions who are unable to judge degree of uncertainty, indicating that the functions of the OFC we are investigating in rats are conserved across species.

These results suggest that a dynamic representation of and access to an internal measure of decision confidence is fundamental to adaptive behavior—even in rats—and provide insights into the nature of the neural processes underlying decision making. Our findings establish neuronal correlates and behavioral assays that lay a foundation for a broader examination of the neural mechanisms underlying confidence estimation.

Neural Mechanisms of Ketamine Psychoactivity in Behaving Rats

M. Quirk, D. Sosulski, C.E. Feierstein, N. Uchida

Ketamine is a psychoactive drug that has been described clinically as mimicking acute psychosis and is considered one of the leading models for schizophrenia. More recently, acute ketamine administration has also been shown to be a promising therapeutic agent for treating intractable depression. Understanding the mechanism

of action of this drug has therefore generated considerable recent interest.

A prominent hypothesis for ketamine function is that it acts to inhibit prefrontal cortical interneurons. This idea links prominent glutamatergic and GABAergic theories of schizophrenia, potentially explaining why this NMDA (*N*-methyl-D-aspartate) receptor blocker results in an increase in frontal glutamate release. Recently, H. Homayoun and B. Moghaddam (University of Pittsburgh) provided support for this "disinhibition" hypothesis by showing that ketamine selectively inhibits putative interneurons in awake rats.

In our work, using multielectrode recordings in the orbitofrontal cortex of behaving rats, we were able to extend these results in several important ways. First, we show that the effects of ketamine are specific to a limited subpopulation of GABAergic interneurons corresponding to FS ("fast-spiking") interneurons (basket and chandelier cells). Second, we show using cross-correlation analysis that, relative to other interneurons, the ketamine-sensitive interneurons preferentially interact with one another and receive greatly enhanced input from local pyramidal neurons. Third, we show that in a behavioral decision-making task, the firing of the ketamine-sensitive population is negatively correlated with response time, a widely used index of motivational state and reward expectancy.

From a translational perspective, these observations provide insight into the mechanism by which ketamine produces its psychoactive effects. From a basic science perspective, they demonstrate the importance of linking in vivo extracellular recordings to in vitro cell-type data and illustrate the specificity of cell function within the cortical microcircuit.

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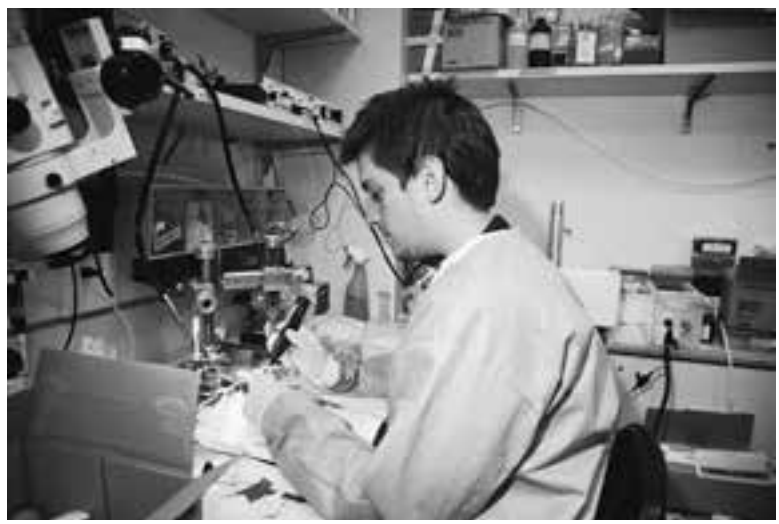

Giullame Dugue

TRANSMISSION AND PLASTICITY IN MAMMALIAN CENTRAL SYNAPSES IN HEALTH AND DISEASE

R. Malinow J. Boehm, N. Dawkins-Pisani, H. Hagiwara, H. Hsieh, H. Hu, H. Kessels, M. Klein, C. Kopec, B. Li, H. Makino, W. Wei

My laboratory is directed toward understanding synaptic function, synaptic plasticity, and synaptic dysfunction. Through such an understanding, we hope to elucidate how learning and memory are achieved and how diseases corrupt them.

This year marks our 14th, and last, year at CSHL. Since our arrival, much has changed in our field. When we arrived, there was still little known about the mechanisms underlying synaptic plasticity. We identified silent synapses, which showed central synapses to be different from peripheral synapses, and helped solve a conundrum regarding long-term potentiation (LTP). We made considerable headway in characterizing α-amino-3-hydroxy-5-methyl-4-isoxazole proprionic acid (AMPA) receptor trafficking and showed it to be an important underlying mechanism in long-term potentiation (LTP). We have further shown that this mechanism of plasticity underlies developmental plasticity as well as certain forms of associative learning. Priming AMPA receptor delivery to synapses occurs during emotional arousal and enhances learning. We have also seen that AMPA receptor trafficking has a key role in the pathophysiology produced by β-amyloid, an agent thought to be causative in Alzheimer's disease. Our more recent studies have identified a synapse that may be abnormally enhanced on "disappointment" neurons and responsible for depression. It has been an exhilarating, although sometimes turbulent, ride. I thank all those at CSHL who have made this possible.

Dendritic and Axonal Secretion of β-Amyloid Reduces Local Spine Number and Plasticity

W. Wei, R. Malinow [in collaboration with S. Sisodia, University of Chicago]

β-amyloid (Aβ), a peptide generated by neurons, is widely believed to underlie the pathophysiology of Alzheimer's disease when overproduced. Recent studies indicate that this peptide can drive endocytosis of AMPA- and *N*-methyl-D-apartate (NMDA)-type glutamate receptors and lead to loss of dendritic spines. We now show that Aβ released from either presynaptic or postsynaptic structures can have this effect. This answers a long-standing question in the field regarding the origin of Aβ responsible for synaptotoxic effects.

PSD-95 Is Required for Activity-driven Synaptic Development

I. Ehrlich, R. Malinow

The activity-dependent regulation of AMPA-type glutamate receptors and the stabilization of synapses are critical to synaptic development and plasticity. One candidate molecule implicated in maturation, synaptic strengthening, and plasticity is PSD-95. Here, we find that acute knockdown of PSD-95 in brain slice cultures by RNA interference arrested activity-driven development of synaptic structure and function, manifested by reduced synaptic strength and altered spine morphology. Surprisingly, PSD-95 was not necessary for the induction and early phase of LTP expression. However, following PSD-95 knockdown, chemically induced LTP produced smaller changes in the size of stable spines, and we observed a larger fraction of transient spines that turned over more readily. Taken together, our data support a model in which PSD-95 is required for activity-dependent synapse stabilization following initial phases of synaptic potentiation.

Abnormally Enhanced Transmission onto Lateral Habenula Neurons Projecting to Ventral Tegmental Area in Congenitally Depressed Rats

B. Li, R. Malinow [in collaboration with F. Henn, Brookhaven National Laboratory]

Increased activity levels in the lateral habenula (LHb) has been observed in humans during depression by

fMRI (functional magnetic resonance imaging) studies. Neurons in the LHb have been prveviously shown to become active and neurons in the ventral tegmental area (VTA) reduce their activity during "disappointment" events in awake behaving monkeys. Here we show that LBh neurons projecting to VTA show a fivefold to tenfold increase in spontaneous miniature excitatory synaptic current (EPSC) frequency in animals bred to be sensitive to a depression model (learned helplessness). These studies suggest that a defect at this synapse is responsible for persistent disappointment underlying depression.

Emotion Enhances Learning via Norepinephrine Regulation of AMPA Receptor Trafficking

H. Hu, E. Real, R. Malinow [in collaboration with J. LeDoux, New York University; R. Huganir, The Johns Hopkins University]

Emotion enhances our ability to form vivid memories of even trivial events. Norepinephrine (NE), a neuromodulator released during emotional arousal, has a central role in the emotional regulation of memory. However, the underlying molecular mechanism remains elusive. Toward this aim, we have examined the role of NE in contextual memory formation and in the synaptic delivery of GluR1-containing AMPA-type glutamate receptors during LTP, a candidate synaptic mechanism for learning. We found that NE, as well as emotional stress, induces phosphorylation of GluR1 at sites critical for its synaptic delivery. Phosphorylation at these sites is necessary and sufficient to lower the threshold for GluR1 synaptic incorporation during LTP. In behavioral experiments, NE can lower the threshold for memory formation in wild-type mice, but not in mice carrying mutations in the GluR1 phosphorylation sites. Our results indicate that NE-driven phosphorylation of GluR1 facilitates the synaptic delivery of GluR1-containing AMPA receptors, lowering the threshold for LTP, thereby providing a molecular mechanism for how emotion enhances learning and memory.

Spine Enlargement Precedes AMPA Receptor Exocytosis during LTP

C. Kopec, B. Li, W. Wei, J. Boehm, R. Malinow

The changes in synaptic morphology and receptor content that underlie neural plasticity are poorly understood. In this study, we used a pH-sensitive green fluorescent protein (GFP) to tag recombinant glutamate receptors and showed that chemically induced LTP drives robust exocytosis of AMPA receptors onto dendritic spines. In contrast, the same stimulus produced a small reduction of NMDA receptors from spines. LTP produced similar modification of small and large spines. Interestingly, during LTP induction, spines increased in volume before surface incorporation of AMPA receptors, indicating that distinct mechanisms underlie changes in morphology and receptor content. We are continuing these studies by establishing optical methods to detect synapses that have undergone plasticity.

Subcellular Distribution of AMPA Receptors Depends on Stargazin, Subunit Composition, and Phosphorylation Events

H. Kessels, M. Klein, R. Malinow

Understanding the fate of synthesized AMPA receptors is important in elucidating mechanisms of neuronal function. For instance, studies have proposed that increased synthesis of AMPA receptors mediates synaptic plasticity. Here, we examine the effect of increased synthesis of AMPA receptor subunits GluR1 and GluR2 on their subcellular distribution. Virally driven expression of AMPA receptor subunits in rat organotypic hippocampal slices results in increased levels in cell bodies but little increase in dendritic regions and no change in synaptic transmission. Stargazin (STG), an AMPA receptor regulatory protein, promotes a large GluR1 redistribution into dendrites. Upon dendritic transport, GluR1 can be protected from lysosomal degradation by binding to STG or GluR1 phosphorylation. However, increased dendritic GluR1 levels do not enhance surface or synaptic levels of GluR1, indicating that simply increasing the production of AMPA receptor subunit GluR1 in dendrites cannot account for a change in synaptic strength. STG also facilitates transport of GluR2 into dendrites but does not protect GluR2 from lysosomal targeting yielding no increase in total or surface levels of dendritic GluR2. Interestingly, expression of a phospho-mimetic mutated STG selectively enhances surface and synaptic GluR2 but not GluR1 levels. Our data show that cellular localization of AMPA receptors is tightly controlled by subunit composition, STG, and phosphorylation events.

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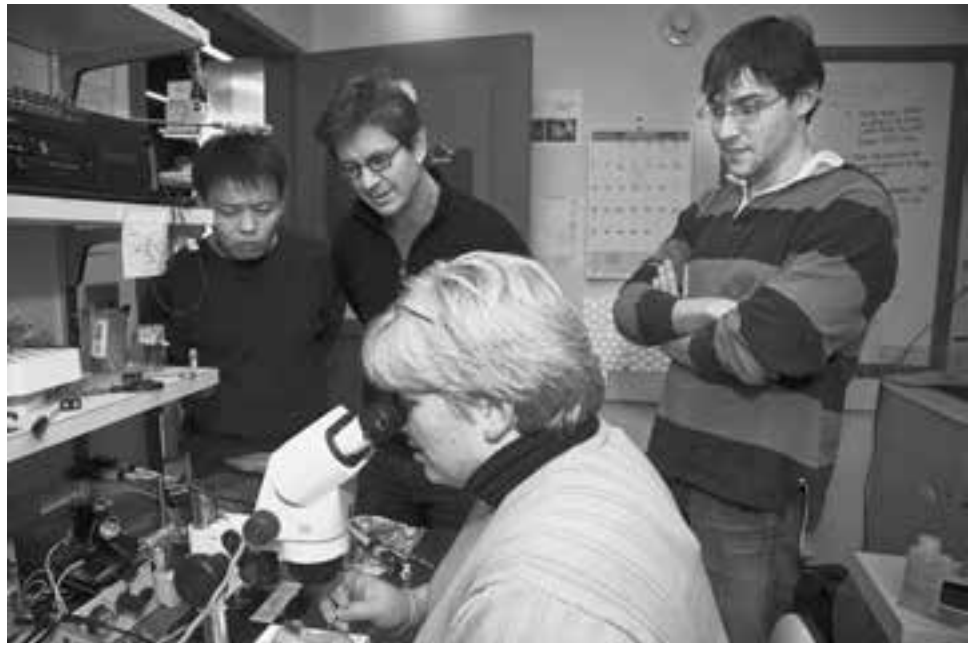

Roberto Malinow (*standing*, *center*) with members of his lab

INTEGRATIVE SYSTEMS NEUROBIOLOGY

P. Mitra P. Andrews J. Bohland H. Bokil J. Lin J. Serkhane D. Valente H. Wang C. Wu

Our basic research philosophy is to combine theoretical, computational, and experimental approaches for the study of complex biological systems, with a particular focus on neurobiological questions. Efforts in our group fall into three main areas: Neuroinformatics, Theoretical Engineering, and Quantitative Behavior and Electrophysiology.

Our neuroinformatics research involves the application of statistical and signal-processing tools to large volumes of neurobiological data as well as the development of informatics infrastructure for large-scale data integration. Our study in the area of theoretical engineering applies theories developed alongside human-engineered systems to study the theoretical principles underlying the design of biological systems. The final area of our research is experimental and consists of behavioral and electrophysiological studies in multiple species including *Drosophila*, zebra finch, macaque monkey, and human infants.

Continuing in the Mitra laboratory in 2007 were Peter Andrews (scientific informatics manager), Hemant Bokil (postdoctoral fellow), Haibin Wang (postdoctoral fellow), Dan Valente (postdoctoral fellow), and Jihene Serkhane (postdoctoral fellow). Joining us in 2007 were Jason Bohland (scientific informatics manager), John Lin (scientific informatics developer), and Caizhi Wu (postdoctoral fellow).

We have ongoing collaborations with multiple research groups here at CSHL and other institutions, which currently include electrophysiological studies in macaques and humans with Nicholas D. Schiff and Keith Purpura at the Weill Medical School of Cornell University, where Dr. Mitra is an adjunct associate professor; a consortium of zebra finch researchers, including a long-standing collaboration with Ofer Tchernichovski at City College of New York (CCNY); study of memory formation in *Drosophila*, together with Josh Dubnau at CSHL; and the Brain Architecture Project, begun in 2006 with co-investigators at Harvard/Massachusetts General Hospital, California Institute of Technology, and the University of Southern California.

Chronux: Open Source Software for Neural Signal Processing

H. Bokil, P. Andrews

Nervous system activity can be measured at many spatial and temporal scales, from electrophysiological recordings of single neurons to monitoring large networks of neurons or brain areas with various imaging technologies. For the last several years, our group has been developing algorithmic and computational tools for the statistical analysis of such neural time series data, and we are encoding these tools into an open source software package entitled Chronux (www.chronux.org). This continuing project includes the development of a high-quality numerical analysis library, data input-output and management utilities, and a user interface that gives experimental neuroscientists access to advanced analysis tools. We expect that these tools will be critical for advancing our understanding of systems-level neuroscience. We have made a number of additions and changes to Chronux during the past year, and we are currently preparing a major release.

We have added the FTrack package to Chronux, which is a video-based behavioral analysis system. FTrack is able to track and record the trajectory of a single animal being videotaped in an open arena. The software is Matlab-based and comes with a GUI (graphical user interface) to load movies, adjust parameters, and track in an interactive environment. In response to user feedback, we have modified the Matlab interface to LOCFIT that is distributed with Chronux. A further addition to Chronux is a data browser GUI which we have been developing for visualization and analysis of time-series data. The GUI is capable of simultaneous display of multiple time series in a raster-like fashion, which is useful for visualizing any type of multichannel data such as EEG (electroencephalography) or MEG (magnetoencephalography). The software is capable of displaying raw voltage traces, as well as derived data such as spectra and spectrograms.

Song Learning in the Zebra Finch

H. Wang

Our principal goals are to provide the informatics infrastructure for the integrative study of zebra finch vocal development, to provide a theoretical framework that brings together the different modalities of experimental data, and to develop signal-processing algorithms for integrating the heterogeneous streams and for hypothesis discovery and testing.

In the past year, progress has been made on the construction of an information infrastructure to effectively handle the multiterabyte data sets generated by the project, including the continued construction of an online storage resource, coupling of this resource to the cluster at CSHL High-performance Computing Center (HPCC) and development of interfaces to semantic Web database technologies. We have also provided the consortium of researchers with a Web portal that is now being actively used to manage digital resources within the consortium, such as weekly meeting notes and electronic laboratory notebooks.

Our approach to signal processing is based on spectral analysis, with appropriate adjustments made to the algorithms to suit domain-specific needs. We studied the characterization of birdsong using spectral correlations. This measure complements the existing methods in two ways: It does not require segmenting a song into syllables, and it is not confined to a short time frame. We used the high-performance computing cluster here at CSHL to study the vocal development process of zebra finch with this measure.

Toward an Integrative Model of Memory in *Drosophila*

D. Valente

Memory is a process spanning many levels of organization from genetics to behavior. One of the major challenges faced in neuroscience today lies in developing functional relationships between the genotype of an organism and its behavioral phenotype, integrating activity at each level. Applied to the problem of memory, such a relationship would span the scales and allow unprecedented insight into how memories are formed and stored. Whereas much *Drosophila* memory research has primarily focused on developing detailed descriptions of either the genetic networks or the neural networks involved in the process, only recently have there been attempts to integrate knowledge at these levels into a single, overarching description of memory. Our project is focused on developing an integrative model of memory, taking into account activity at each level of organization.

In light of this, the past year has focused on the development of two experimental setups, both of which elucidate processes at the behavioral level and expand the quantitative behavioral phenotype of wild-type flies. The first setup is a flight simulator for tethered-flight conditioning assays of *Drosophila* (seen in Fig. 1). Numerous studies have shown that flies can be conditioned in this paradigm to avoid certain patterns presented to them in their visual environment. In collaboration with Michael Dickinson's lab at the California Institute of Technology, we have attempted to replicate these well-documented visual learning studies. Although neither our lab nor the Dickinson lab has been able to observe the learning reported in the literature, analysis of our results is beginning to show subtle, yet important, differences between our setup and the setup used in published studies. This seemingly "failure" to replicate has turned out to be quite fortuitous, as it has led to further investigation of the importance of precisely timed feedback in this assay and has initiated an investigation of the avoidance response of flies to an intense heat stimulus. The avoidance response has not been systematically studied in the literature, but it is required if one is to properly interpret the results of any aversive conditioning assay in the simulator. In all, this research is leading to a greater understanding of learning and memory in this paradigm.

Similarly to studies of flies in flight, measuring locomotor behavior of *Drosophila* in enclosed arenas is a powerful way to obtain a quantitative behavioral phenotype. In collaboration with Ilan Golani of Tel Aviv University, we have been studying the locomotor

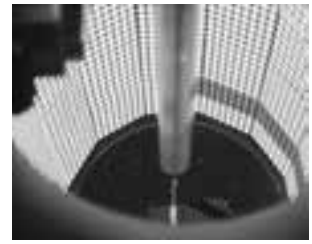

FIGURE 1 The flight simulator in action.

behavior of flies in the Open Field and have developed quantitative measures to describe this behavior. The majority of the measures previously used for this purpose are relatively coarse: They either are based on average velocities, thus ignoring fine-scale movements of the fly, or simply count the number of times the fly crosses a point on a linear track.

We have developed a video-tracking system to acquire the trajectory of a fly over long timescales as it explores a circular open-field arena and developed several metrics that take into consideration the dynamics of the entire trajectory, as well as the interactions of the trajectory with the geometry of the arena. These metrics are all based on probability distributions of the fly's behavior and can be worked into nearly any behavioral setup. In addition, they are relatively easy to calculate and can account for environmental effects on the behavior. Such metrics complement those that are currently used to describe the locomotor behavior of *Drosophila*, thus expanding the behavioral phenotype for flies in this assay.

The Brain Architecture Project

J. Bohland, H. Bokil, J. Lin, C. Wu

Although the entire genomes of many species including human have been sequenced, our understanding of the architecture of the human nervous system, in terms of the organization of its connections, remains crude. The long-term objective of the Brain Architecture Project, funded by the W.M. Keck Foundation, is to produce a draft of the mesoscopic connectivity matrix for the human brain as well as associated tools for visualization and analysis. The project, which was in its first full year in 2007, will initially focus on extracting the available knowledge about human neuroanatomical connections from the existing literature and making these data available to scientists and clinicians through a unified online knowledge base. In 2007, a major focus was setting up the infrastructure, designing appropriate data models, and assembling a processing pipeline for machine-assisted literature curation. The design of this workflow mirrors those in place in the bioinformatics community, which have largely been absent in neuroscience.

Any attempt to assemble a neuroanatomical connectivity graph must, almost immediately, confront the problem of how to partition the brain into a set of appropriate nodes or regions and how to label those regions. Because multiple such schemes and naming conventions appear in the extant literature, it is vital to have an understanding of how these systems relate to one another and to develop a strategy for reconciling the different systems within a common framework in our project. Brain Architecture Project researchers have developed an analytical spatial framework within which such different parcellation schemes can be quantitatively compared and have applied these methods in a comparison of different digital atlases used in the neuroimaging community. The results reveal a surprising lack of concordance between atlases and point to the inadequacies of previous efforts to address the neuroanatomical nomenclature problem. These procedures instead provide probabilistic mappings between regions from different atlases, which will facilitate and improve the accuracy of cross-study comparisons. These results and methods will be made available as an online tool in 2008.

The Brain Architecture Project also promises to deliver tools for graph-theoretical analysis that can be applied to the draft connectivity matrix. This year, project members used graph-theoretical analyses to investigate a different network, that which represents the greater neuroscience community, as constructed from a database of scientific abstracts presented at the Society for Neuroscience meetings from 2001 to 2006. Also using tools from text mining, the structure of the neuroscience community was analyzed from these abstracts, revealing community demographics, topic clusters, the structure of funding sources, and the dynamics of the field over time.

The first annual Brain Architecture Project meeting was held May 20–22, 2007, at the Banbury Center. The meeting was attended by many distinguished neuroscientists, anatomists, and clinicians, and discussion was focused on the so-called nomenclature problem that

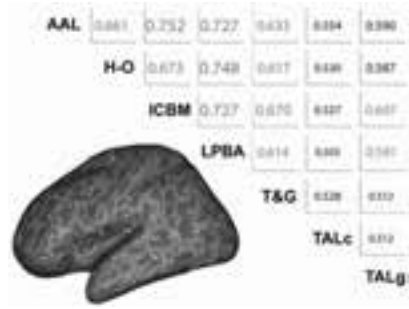

FIGURE 2 Comparison of human brain parcellation schemes. (*Lower left*) The boundaries of two parcellations are drawn on the same inflated cortical hemisphere. (*Top right*) The similarity between eight different schemes as measured by an index created in the lab, with above chance similarities in gray.

exists in the delineation and naming of brain regions, as well as the general need for experimental programs to systematically map the mesoscopic connectivity patterns in model species.

MISCELLANEOUS

This year saw the publication of a book by Drs. Mitra and Bokil (*Observed Brain Dynamics*). This caps a decade of work in the area of neural signal processing and should provide a textbook suited to educating neuroscientists in advanced data analytical methods. Dr. Mitra was codirector of two summer courses, the Neuroinformatics course at the Marine Biological Laboratories and the Integrative Analysis of High-throughput Biological Data at CSHL. He also organized two Banbury meetings, Design Principles in Biological Systems (the second of such annual meetings) and the first annual meeting of the Brain Architecture Project.

Finally, Dr. Mitra was instrumental in putting together a series of public science lectures entitled *Science Soirees* at the Science, Industry, and Business Branch of the New York Public Library. This series of lectures, which featured speakers from CSHL as well as elsewhere talking about scientific topics of general interest to a broad audience, was well attended and received coverage in the *Village Voice*.

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John Lin

GENE-COPY-NUMBER VARIATION AND ITS ROLE IN HUMAN DISEASE

J. Sebat M. Kusenda D. Malhotra
P. Lin S. McCarthy
V. Makarov S. Yoon

Large-scale differences in gene copy number called copy number variants (CNVs) are a significant source of human genetic variation. In contrast to DNA sequence variants such as single-nucleotide polymorphisms (SNPs) and microsatellite repeats, CNVs have not been well-characterized. We seek to understand more about the genomic locations, frequency, and stability of these structural variants and their role in human disease.

Determining the Genetic Basis of Autism

J. Sebat, S. Yoon, M. Kusenda, P. Lin, D. Malhotra [in collaboration with M. Wigler, K. Ye, B. Lakshmi, M. Ronemus, and A. Krasnitz, Cold Spring Harbor Laboratory]

Our work on the genomic analysis of autism has made distinct contributions to our understanding of the genetic basis of cognitive disorders. We have shown that microarray-based CNV analysis can detect copy-number mutations at a much higher resolution than cytogenetic methods, and we have used these methods to identify a strong association of de novo copy-number mutations with autism (Sebat et al. 2007). In the latter study, we showed that spontaneous CNVs are frequent in sporadic autism (10%), a rate much higher than in healthy individuals, suggesting that the majority of mutations that we detected contribute to the disorder. In contrast, spontaneous mutations were not significantly associated with disease in families with more than one affected child, indicating that in terms of the genetic mechanism involved, there is a distinction between the familial and sporadic cases.

The mutations that we detected occurred at many loci throughout the genome and few were detected more than once. These results are consistent with the findings of linkage studies and cytogenetics and support the hypothesis that there are many genes in the genome that potentially contribute to autism spectrum disorder (ASD), with each gene contributing to only a small fraction of cases. Our findings implicate specific genes in autism, including genes involved in sterol metabolism, hormonal signaling, and synaptic transmission; however, further studies are needed to determine the contribution of each gene to the disorder in the broader population of patients. To this end, we have begun candidate gene studies, including sequencing and fine-scale CNV analysis in a large sample of patients and controls. Preliminary results suggest that more complete ascertainment of variation within candidate regions may reveal the greater extent of an individual gene's contribution to ASD. For instance our recent work done in collaboration with Dan Geschwind (University of California, Los Angeles) has found a convergence of evidence from analysis of SNPs and CNVs implicating the gene *CNTNAP2* in ASD.

A Genomic Analysis of Schizophrenia and Bipolar Disorder

J. Sebat, S. McCarthy, S. Yoon, P. Lin [in collaboration with S. Gary, J. Watson, and T. Leotta, Cold Spring Harbor Laboratory]

A high spontaneous rate of structural mutation is a property of the human genome and exists independently of the phenotypes that emerge from it. Thus, our findings in ASD may serve as a model for other disorders. We hypothesize that rare highly penetrant mutations, including a significant proportion that are CNVs, may also have a role in the etiology of schizophrenia (Scz) and bipolar disorder (BD). We have therefore recently initiated genetic studies of Scz and BD, with a focus on families with sporadic and young-onset cases. In our study of Scz, we have performed CNV analysis of 1200 patients and 1300 healthy controls. We have found strong evidence for CNV risk factors at several loci in the genome. Some of the mutations that we have identified occur relatively frequently among patients (~0.5%) and quite rarely in healthy individuals (<0.01%). In addition, we examined the total abundance of rare CNVs among patients and found that novel deletions and duplications of genes were present in 15% of patients compared to 5% of controls ($P = 0.0008$). The abundance of rare variants was higher still (20%) among young-onset cases ($P = 0.0001$). The association was independently replicated in patients with childhood-onset schizophrenia ($P = 0.017$). In addition, we observed spontaneous mutations

in about 4% of patients. Mutations in cases disrupted genes disproportionately from signaling networks controlling neurodevelopment, including neuregulin and glutamate pathways. These results suggest that multiple, individually rare mutations implicating genes in neurodevelopmental pathways contribute to schizophrenia.

A Case Control Study of Parkinson's Disease

J. Sebat, D. Malhotra, S. Yoon

Parkinson's disease (PD) is the most common neurodegenerative movement disorder characterized by age-dependent increases in bradykinesia, muscular rigidity, gait abnormalities, and a rest tremor. PD can arise from multiple etiologies, including genetic mutations that for the most part are uncommon. Evidence has been lacking for the existence of common genetic variants with large effect, but it does exist for multiple genes including common alleles with small effect and rare highly penetrant alleles. Such complex etiology is inherently difficult to study using traditional genetic approaches. Analysis of genome copy-number polymorphisms holds great promise in identifying structural variants predisposing to PD pathology. We used an 85K probe representational oligonucleotide microarray analysis (ROMA) to screen 300 late-onset sporadic PD cases and an equal number of matched controls from Caucasian and Chinese cohorts. Findings include the detection of deletions in both familial and sporadic cases involving two genes, *ZIC1* and *ZIC4*, which are related to cerebellar development. Also detected were deletions of *Parkin*, a gene previously implicated in recessive early-onset PD. These findings suggest that heterozygous deletions of *Parkin* may also have a role in late-onset PD. More comprehensive studies of candidate genes identified in this study will help us to determine the contribution of these genes to the disease.

Population-based Studies of Copy-number variation

S. Yoon, M. Kusenda, D. Malhotra, V. Makarov [in collaboration with K. Ye and C. Lee, Cold Spring Harbor Laboratory]

Much remains to be learned about the extent of "normal" structural variation in the human genome and its implications for genetic studies of populations. We have initiated a CNV-discovery effort that will focus on DNA samples from the international HapMap project. The key advantage of using HapMap samples for this study is the availability of high-density SNP genotypes on the same individuals. CNV data can be combined with SNP data to determine, for example, whether a polymorphic duplication or deletion is associated with a unique SNP haplotype (indicating that the variant results from an ancestral event that was inherited by descent) or with many different haplotypes (suggesting recurrent structural mutation at that site). In addition, direct analysis of copy-number variation is helpful for resolving aberrant patterns in the HapMap data that result from CNVs, such as apparent non-Mendelian patterns of inheritance. Here, we used a cluster-analysis-based method to detect and genotype common CNVs (minor allele frequency ≥0.1) from microarray intensity data on a large population (N = 2355). Single-oligonucleotide probes were sufficient to detect novel CNVs, enabling us to map common variants without bias in terms of size. A majority of the more than 500 common CNVs identified in this study were small and had not been previously detected in humans or in the same data set using a standard algorithm for CNV discovery. These results led us to estimate that approximately 2000 CNVs (>1 kb) are common in this population.

Common duplications and deletions were gene-poor, containing fewer exons on average than CNVs in general. We determined the distribution of CNVs in East Asian, Caucasian, and African American populations and examined the association of CNVs with SNP-based haplotypes. Interestingly, some CNVs were associated with haplotypes showing evidence of recent positive selection in humans. We hypothesize that some common CNVs identified here have phenotypic effects that have been favored by natural selection.

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NEUROGENETICS OF MEMORY IN *DROSOPHILA*

T. Tully K. Bell, F. Bolduc, Y. Chen, H. Cox, Y. Hua, W. Lin, M. Regulski, N. Sinha, H. Wang, S. Xia

Studies on Pavlovian learning in many animals have revealed remarkably similar behavioral properties of associative learning. Surprisingly, Pavlovian learning in fruit flies also displays these behavioral properties, suggesting a common underlying mechanism. Neural architecture of the *Drosophila* brain bears no resemblance to that of mammalian brains, of course, which suggests that the behavioral "homology of function" must result from conserved cellular/molecular mechanisms. Consistent with this view, several genes and genetic pathways have been identified to function in both vertebrate and invertebrate associative learning, including *N*-methyl-D-aspartate (NMDA) receptors, cAMP signaling, cAMP response element binding (CREB)-dependent gene transcription, and staufen- and fragile-X mental retardation protein (FMRP)-mediated local regulation of protein translation. This systematic, rationale approach now is beginning to connect the biochemistry of memory formation with the molecular bases of heritable forms of mental retardation. Translational efforts (in collaboration with Helicon Therapeutics, Inc.) also have generated two small-molecule drugs in clinical trials and a drug development pipeline with six novel programs.

MUTANTS AND MICROARRAYS

We continue work derived from earlier experiments on regulated gene expression induced during memory formation. We also continue to develop novel analytical methods using "concordance" between fully replicated experiments as the guiding metric.

We are also "connecting the dots" among various memory mutants. Over the years, we have systematically moved down the cAMP pathway, from G protein to adenylyl cyclase, to cAMP phosphodiesterase, to cAMP-dependent protein kinase (PKA), to CREB protein, to *staufen, pumilio*, and *dFMR*. In collaboration with Drs. M. Zhang and J. Dubnau here at CSHL, we also have applied genomic informatics to identify *discs large* (*dlg*) as a target of *pumilio* function (see Publications). Dr. S. Xia in our lab has also taken a closer look at G-protein-mediated signaling in olfactory behavior. By developing four new assays for (1) spontaneous response to odor intensity (quantity), (2) spontaneous response to odor identity (quality), (3) conditioned response to odor intensity, and (4) conditioned response to odor identity, Dr. Xia has observed a genetic and anatomical dissection of olfactory processing. Of note, Pavlovian discrimination learning appears to alter odor preference behavior not by changing the fly's perception of odor identity, as expected, but rather by changing the fly's perception of odor intensity. This observation suggests that cell-type-specific wiring of mushroom body calyx by projection neurons from the antennal lobe will determine the perception of odor quality, whereas changes in synaptic strength within calyx microdomains will alter the perception of odor intensity.

CIRCUITS AND PHASES

We continue spatiotemporal experiments, which reveal where in the adult brain long-term memory (LTM) formation occurs. This year, we have shown that modulations of the NMDA receptor subunit genes, *NR1* and *NR2*, restricted to adult mushroom bodies do not affect one-day memory after spaced training. In a region outside of the mushroom bodies referred to as the ellipsoid body, however, these same modulations can either disrupt or enhance LTM. These data suggest that NMDA-receptor-dependent memory formation requires anatomical

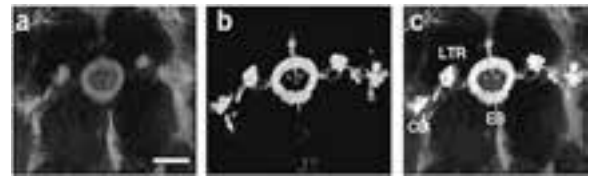

FIGURE 1 dNR2 protein is expressed in the ellipsoid body of the adult *Drosophila* brain. (*a*) Immunostaining for dNR2 showing preferential (but not exclusive) expression of dNR2 protein in cell bodies and neuropil of neurons comprising a subset of the ellipsoid body, which itself is an anatomical compartment of the central complex. (*b*) *Feb170* enhancer-trap-driven expression of green fluorescent protein (GFP). (*c*) Merged image of *a* and *b*, revealing cells with a common expression.

structures in the adult fly brain in addition to the mushroom bodies. We speculate that persistent neural activity from mushroom bodies is required to drive the memory consolidation process in ellipsoid body in an NMDA-receptor-dependent fashion (i.e., LTM requires glutamatergic transmission).

GENETIC MODELS OF DISEASE

We have succeeded to demonstrate an acute (biochemical) requirement for FMR1 during LTM formation in flies. Pharmacological study of *fmr1* mutants also has begun to establish the molecular mechanism by which FMRP functions during behavioral (and presumably synaptic) plasticity. Following on from this work, we more recently have shown that LTM specifically is blocked in *dube3a*^{15B} mutants. This gene is the fly homolog of ubiquitin ligase E3A (UBE3A), which has been shown to underlie some cases of Angelmann syndrome in humans. Thus, we extend our fly models of mental retardation.

We also have developed an oxidative stress-enhanced model Parkinson's disease in flies and currently are pursuing DNA microarray experiments to identify potential genetic enhancers and suppressors of this disease state.

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NEURAL CODING IN THE *DROSOPHILA* OLFACTORY SYSTEM

G. Turner R. Campbell
E. Demir

How are different sensory stimuli represented distinctly in neuronal spike trains? This classic problem in neuronal coding is particularly challenging in the olfactory system because of the vast chemical diversity of odors. Our goal is to understand how odors are represented in terms of spike trains and how learning modifies those representations.

To represent the large number of odors, animals have evolved a large family of olfactory receptors (ORs). There are roughly 300 different ORs in humans, 1000 in rodents, but only 61 in *Drosophila*, making the prospect of understanding olfactory coding much more feasible in this animal. In *Drosophila*, we can electrophysiologically characterize odor response properties of neurons at several successive layers in the olfactory pathway, starting in the sensory layer, up through areas of the fly brain that are important for learning and memory. In fact, only two synapses downstream from the sensory layer is a brain area called the mushroom body (MB) that is essential for learning and memory. Together with the genetic techniques available to identify neurons and to manipulate and optically record their activity, and the learning and memory mutants isolated by behavioral genetics, this constitutes an excellent experimental system to study learning at multiple levels.

Our long-term goal is to understand how information from two different sensory streams is bound together in the brain when a fly learns that a particular odor is paired with an aversive or appetitive taste. What changes in neural activity underlie this learning? What synapses change strength? What ion channel properties change? How do these changes alter cellular and population-level stimulus representations so that the fly's avoidance response to the aversive taste is transferred to the odor?

Odor Representations in Mushroom Body Neurons

G. Turner

Odors typically activate several different olfactory sensory neurons, and it is thought that odor identity is encoded combinatorially by an ensemble of coactive sensory neurons. A highly combinatorial coding scheme is also prominent at the second layer of the pathway, where neurons also respond to many odors. Although dense combinatorial coding provides the first two layers of the network with a high capacity to represent many different stimuli, there are drawbacks to this format. When coding is dense and distributed, an individual neuron participates in the representation of several odors. Consequently, synaptic changes of that neuron will alter the representation of many odors, not just one, a problem referred to as synaptic interference.

To investigate how this problem is resolved, we characterized odor responses in MB neurons, the next layer of the pathway. In contrast to the first two layers of the network, MB neurons are highly odor selective. We found that representations of different odors were less correlated in the MB neurons than in the sensory neurons, indicating that synaptic interference would indeed be diminished in the MB. However, there is a trade-off between stimulus selectivity and the loss of information: In the extreme case of an MB neuron that responds to only a single odor, the problem of synaptic interference is solved; however, information about all other odors has been lost to downstream layers of the network. One way to preserve this information is if individual MB neurons receive input from a variety of PN (projection neuron) types from different glomeruli. Combining input from different PN types would enable the number of different MB tuning curves to expand combinatorially. This diversity might ensure that, even though individual MB neurons respond to few odors, a given odor would likely evoke a response somewhere in the population of 2500 MB neurons.

We are testing these possibilities by (1) directly measuring the convergence of different inputs onto MB neurons, (2) assessing the diversity of MB odor tuning curves, and (3) using behavioral methods to determine whether this convergence endows the animal with odor discrimination capabilities that cannot be attributed to an individual sensory input channel.

Stimulus Selectivity and Memory Formation

E. Demir

Using a mixture of genetics, behavior, and electrophysiology, we plan to directly test the hypothesis that the high stimulus selectivity of MB neurons underlies the specificity of olfactory memory. Our first goal is to establish a behavioral measure for the accuracy of olfactory memory formation and retrieval. Using a panel of chemically similar odorants, we plan to test how effectively flies can learn to discriminate these odors. This psychometric measure of olfactory similarity is an essential starting point for studying how olfactory representations change during learning.

Using the genetic tools available in *Drosophila*, we can manipulate sparseness levels and test the consequences on accuracy of memory formation and retrieval. Decreasing odor selectivity is predicted to make memory recall less accurate, whereas increasing selectivity may increase the accuracy of learning, although there may be a corresponding decrease in the capacity to learn multiple associations.

We are also planning to determine how odor representations change in the cells postsynaptic to the MB neurons associative conditioning. MB output is required for memory retrieval, strongly suggesting that the activity of these neurons will be modified by learning. We aim to determine whether perceptual distance between different odors is reflected in the spiking responses of the MB output neurons.

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Ebru Demir

NEURAL CIRCUITRY OF UNDERLYING NORMAL AND ABNORMAL PROCESSING IN THE CORTEX

A. Zador H. Asari, T. Hromadka, S. Jaramillo, S. Lima-Mainen, G. Otazu, H. Oviedo, L.-H. Tai, 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. We use a combination of theoretical and experimental approaches to study how the brain computes. Experimental approaches include electrophysiological and imaging techniques at the molecular, synaptic, cellular, circuit, and behavioral levels.

Context Dependence and Response Predictability in Rat Auditory Cortex

H. Asari

Sensory signal processing in the brain depends on stimulus history and contexts. Classical linear encoding models with rather shorter timescales (typically, hundreds of milliseconds), however, have failed to fully capture neural dynamics in the auditory cortex. Here, we used whole-cell recordings in vivo to assess the relevant timescales and how neural responses depend on the stimulus context. We found that the changes in lower-order sound properties (e.g., intensity) had larger and longer effects than the changes in higher-order properties (e.g., amplitude-modulation), and the context dependence sometimes lasted as long as 4 seconds in some neurons. We also showed that the predictive power for the best nonlinear model, but not for the linear models even with static nonlinearities, asymptotically approached the predictable response power on a timescale of seconds. These results suggest that complex modulations on longer timescales should be considered as well for fully understanding the auditory cortical activities and functions.

Disruption of Auditory Cortical Circuits by Autism Candidate Genes

T. Hromadka

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 vivo whole-cell patch-clamp methods to compare the ratio of excitation and inhibition elicited by auditory stimuli in single neurons 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.

Identifying Neurons with Channelrhodopsin-2 during In Vivo Recording

S. Lima, T. Hromadka

Neural circuits consist of a heterogeneous mixture of neurons with different neuroanatomical projections and patterns of molecular expression. Recordings of neural activity in behaving animals reveal tremendous functional heterogeneity as well: Nearby neurons often respond very differently to the same stimulus or action. However, little is known about how this structural circuit-level heterogeneity contributes to function, in part because of the technical difficulty of identifying neurons during in vivo recordings in behaving animals.

To overcome this difficulty, we have developed a technique that allows us to "tag" subpopulations of neurons for identification during in vivo electrophysiological recordings. The tag is a light-gated ion channel—the algal protein channelrhodopsin-2 (ChR2)—whose expression can be genetically restricted to a subpopulation of neurons. In the subpopulation of neurons expressing ChR2, a brief flash of blue light triggers a single action potential with millisecond precision.

We are using this approach to test the hypothesis that neuroanatomical connectivity represents one important structural correlate of the functional diversity in the rodent cortex. To do this, we restrict ChR2 expression to subsets of neurons in the rat auditory cortex (ACx). ACx pyram-

idal neurons project to multiple brain regions, including the amygdala, the posterior parietal cortex, and the contralateral ACx, and presumably carry different information about auditory stimuli to these centers. To target ACx neurons specifically based on their projection pattern, we inserted the ChR2-coding region into a herpes simplex virus (HSV). The HSV travels in a retrograde fashion through the axons of infected neurons. ChR2-tagged neurons, i.e., neurons projecting to the infected area, can be identified by their low-latency and reliable spiking response to a brief light flash. Thus, for example, we have used this approach to identify the subpopulation of layer-5 ACx neurons that project to the contralateral cortex.

This approach is general, in that any population to which expression of ChR2 can be genetically restricted can be tagged. Promising future applications include tagging of different subpopulations of neurons based on promoters (e.g., for subclasses of inhibitory interneurons) and tagging of neurons in different cortical layers.

Separation of Sound Sources by Awake Behaving Animals

T. Hromadka, G. Otazu, L.-H. Tai

Sounds in the natural world rarely occur in isolation, but rather as part of a mixture. To survive, the auditory system must be able to attend selectively to one sound source and ignore others—and it does so more effectively than any artificial system yet devised. To understand how this is performed, we are using multielectrode recording (tetrode) technology to monitor the activity of many neurons simultaneously in awake, behaving rodents performing an auditory discrimination task (Hromadka and Zador 2007; Hromadka et al. 2008).

Mapping of Auditory Cortex Circuitry Using Laser-scanning Photostimulation

H. Oviedo [in collaboration with I. Bureau and 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 to that of other sensory cortices, such as the better-studied barrel cortex. Preliminary 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.

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 behavioral 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. 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-choice alternative 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 initial experiments indicate that the cortex can make use of information on a timescale as short as 10 msec, and possibly much less.

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NEURAL BASIS OF LEARNING AND MEMORY IN *DROSOPHILA*

Y. Zhong H.-C. Chiang M. Pagani
I. Hakker Y. Wang
I.S. Ho C. Xu
A. Mamiya

We combine genetic and functional analyses in the study of the neuronal and molecular bases of learning and memory in *Drosophila*. We are involved in three projects to identify new molecular and neuronal events that are essential in learning and memory. One project is related to the normal and pathological function of the gene *corkscrew* which is associated with Noonan syndrome. This syndrome shows a number of developmental defects as well as behavioral alterations. In particular, we are studying the effect on behavior of point mutations associated with the Noonan syndrome. In the second project, we are studying the roles of the Aβ42 peptide associated with Alzheimer's disease in the modulation of synaptic transmission and plasticity in *Drosophila*. The third project is related to the integration of sensory information in the mushroom body, a higher olfactory center that is essential for olfactory associative learning. In this project, we are examining the functional imaging of airflow response in the *Drosophila* mushroom body.

Role of the *Drosophila* Gene *corkscrew* in Long-term Memory: New Insight into the Pathogenesis of Noonan Syndrome

R. Pagani, I. Ho

Noonan syndrome is a dominant genetic disorder showing developmental defects, high incidence of leukemia, mental retardation, and behavioral disorders. Recently, it has been identified that missense mutations in the *PTPN11* gene, which encodes the protein tyrosine phosphatase nonreceptor 11, SHP-2, are associated with Noonan syndrome as well as several types of leukemia. It is thought that gain-of-function mutations are associated with both Noonan syndrome and leukemia. The SHP-2 protein is highly conserved in metazoans and has an essential role in development through growth factor and integrin signaling pathways. However, the genetic and molecular mechanisms that mediate behavioral alterations in Noonan syndrome have not been explored.

To examine the role of SHP-2 in behavior and the underlying mechanisms, we began studying transgenic *Drosophila* flies carrying point mutations associated with Noonan syndrome, leukemia, or both disorders in the *Drosophila* orthologous gene, *csw*. We have found that gain-of-function mutations associated with Noonan syndrome, leukemia, or both disorders may mediate learning and memory defects. Preliminary studies showed that learning and memory defects are not dependent on developmental alterations in the nervous system. Although the csw function was involved in learning and long-term memory, the mechanisms involved seem to be different. For example, learning was affected by an increase in the dosage of the csw gene, but not by missense mutations, whereas long-term memory seems to be more sensitive to the mutation than the dosage of the gene.

In addition, a common behavioral property in invertebrates and vertebras, including humans, is that multiple sessions of learning spaced in time promote the formation of a more enduring memory. SHP-2 seems to be associated in long-term memory formation and regulating the time required to induce this memory during spaced sessions of training. We are currently examining the molecular and cellular bases of the role of *csw* in memory formation in normal, mutant, and transgenic flies. Further genetic, behavioral, and physiological studies will improve our understanding of the role of this gene in normal and pathological behavior.

Distinct Roles of Aβ42 Oligomers and Fibrils in Modulation of Synaptic Transmission and Plasticity Revealed through Genetically Targeted Expression in *Drosophila*

H.-C. Chiang, I. Hakker

Aggregation of amyloid-β-42 (Aβ42) peptides has been suggested to be a primary cause of Alzheimer's disease (AD). To determine how endogenously secreted Aβ42 aggregates regulate synaptic functions under physiological conditions, we examined the effects of expression of Aβ42 targeted to the secretary pathway at the body-wall neuromuscular junction of *Drosophila* larvae. Such expression in the *Drosophila* adult brain leads to age-dependent accumulation of Aβ42 deposits, memory loss,

and neurodegeneration. Voltage-clamp recordings of synaptic transmission and optic analysis of vesicle recycling at presynaptic terminals consistently show that expression of Aβ42 in neurons leads to a reduction of neurotransmitter release. In contrast, expression of Aβ42 in postsynaptic muscle cells elicits an enhancement of neurotransmitter release. Both effects are neutralized by incubation with an antibody against Aβ peptides, suggesting a role for secreted Aβ42 peptides. Concomitantly, application of exogenously prepared Aβ42 oligomers and fibrils elicited opposite effects, with oligomers reducing but fibrils enhancing synaptic transmission.

Additional pharmacological studies and analysis of long-term depression led us to the following conclusion: Aβ42 peptides secreted from neurons act primarily as oligomers that inhibit neurotransmitter release and exert no effect on long-term depression. In contrast, fibrils are major components secreted from muscle cells that enhance synaptic transmission and long-term depression. Thus, different types of cells may secret distinct forms of Aβ42 aggregates, leading to fundamentally different modulation of synaptic functions.

Functional Imaging of the Airflow Response in the *Drosophila* Mushroom Body

A. Mamiya, Y. Wang, C. Xu

The *Drosophila* mushroom body (MB) is a higher-order olfactory center that is essential for olfactory associative learning. It is thought to receive inputs from multiple sensory modalities and associate them to form memories. However, although olfactory input to the MB is well-studied, less is known about inputs from other sensory modalities. As an initial step in understanding how the MB integrates various sensory information, we are studying how the MB neurons respond to a simple airflow stimulus.

We presented a very weak airflow to the antenna of intact flies and monitored the activity of MB neurons in vivo using two-photon calcium imaging. To monitor the activities of different types of MB neurons, we used the Gal4-UAS system to target the expression of the genetically encoded calcium sensor GCaMP in specific neurons. We found that a weak airflow can evoke strong responses in MB neurons, suggesting that the airflow stimulus comprises an important part of the input to the MB. The input region of the MB responded only to airflow *on*, whereas some output regions responded to both the airflow *on* and the airflow *off*, the airflow suggesting possible functional processing of airflow information within the MB. We also found that different types of MB neurons respond with different dynamics to the same airflow stimulus. Furthermore, we identified a functional subdivision within a single type of MB neurons, suggesting a functional organization of the MB at a much finer scale than previously thought.

We are currently extending the study to investigate how olfactory and airflow information interact in different types of MB neurons to better understand how these neurons represent, integrate, and associate different sensory information.

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PLANT GENETICS

The plant group at CSHL is carrying forward the pioneering spirit of Nobel Prize–winning work done at the Laboratory by Barbara McClintock in the 1940s and 1950s. Uncovering the molecular processes that govern how plants grow and develop is of fundamental biological interest and importance, with implications not only for agriculture, but also for human health. CSHL is part of global consortia of researchers involved in the comprehensive sequencing of the *Arabidopsis* and maize genomes and leads the way in studying RNA interference, a naturally occurring gene-silencing mechanism, in the context of plant models.

David Jackson's lab studies genes and signals that regulate plant growth and architecture. This year, they identified a gene that controls how plant cells communicate through small channels, called plasmodesmata. Jackson's lab also continues to identify maize genes with novel roles in development, including, this year, those that control branching, stem cell proliferation, and leaf growth. One of these, *TILLERED1*, encodes a transcription factor that controls the growth of branches and, thereby, the overall shape of the plant. This gene is particularly interesting because it responds to light and may provide a way in which the plant responds to environmental conditions. Jackson and colleagues also have begun to assemble a collection of fluorescent-protein-tagged maize lines that promises to enhance maize genetics research through characterization of developmental gene pathways and identification of maize promoters that can be used in crop improvement.

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 study genes active in the meristem. They have identified a protein complex that suppresses stem cell fate during organ development. This complex includes the chromatin-remodeling factor HIRA, an epigenetic regulator that controls stem cell identity, whose molecular mechanism the lab seeks to elucidate. They also study the role of small regulatory RNAs as potential signals. In *Arabidopsis* and maize, they have shown that polarity in leaves is specified through a novel patterning mechanism involving a cascade of opposing small RNAs.

Plants and animals have evolved to make complex, multicellular organisms from single cells. But plants grow in a way that is wholly distinct. The laboratory of Wolfgang Lukowitz studies genesis of the plant "body-plan"—the molecular steps that regulate cell-fate decisions—and the impact of these decisions on tissue specialization. Pathways important in the plant embryo operate throughout the plant's life cycle, including those that regulate the shoot meristem and maintain the stem cell population and the ratio between differentiating and nondifferentiating cells. This raises the questions: What, then, is special about the embryonic stage? What happens at the very beginning to establish polarity or the coordinates of the body plan before other programs take over? These questions are at the focus of Lukowitz's current research.

Epigenetic mechanisms of gene regulation—chemical and conformational changes to DNA and the chromatin that bundles it—have an important impact upon genome organization and inheritance and have a role in the specification and maintenance of cell fate. Robert Martienssen's lab investigates epigenetic mechanisms involved in gene regulation and stem cell fate in yeast and model plants including Arabidopsis and maize. They have shed light on a phenomenon called position-effect variegation, associated with plant color diversity and caused by inactivation of a gene positioned near densely packed chromosomal material called heterochromatin. This year, Martienssen discovered that "slicing," a central mechanism of RNA interference, is implicated in position-effect variegation. His lab also published data suggesting far more variation in wild populations of *Arabidopsis* than previously recognized, which they attribute to epigenetic modifications of chromosomes, not changes in DNA sequence.

CELL-FATE DECISIONS IN THE EARLY PLANT EMBRYO

W. Lukowitz A. Alexander, M. Bayer, L. Courtney, M. Galli, T. Nawy, S. Peters, J. Williams

In most plants, the early stages of embryogenesis follow a predictable sequence of cell divisions and cell-shape changes reflecting the coordinated fate decisions that lay down the reference points of the body plan. Our goal is to identify and understand the genetic network regulating this process. We are working with the small weed Arabidopsis, an inexpensive experimental model with good comparability to economically more important plants.

Paternal Control of Embryonic Patterning

W. Lukowitz, M. Bayer, M. Galli

Plant development begins with an asymmetric division of the zygote. This division entails a fundamental fate decision that sets the stage for all subsequent patterning events: The small apical daughter cell will produce the proembryo, whereas the large basal daughter cell will mainly form the extraembryonic suspensor. The mitogen-activated protein kinase kinase (MAPKK) gene *YDA* promotes suspensor fate in the basal cells. Loss of *YDA* activity essentially eliminates formation of the extraembryonic suspensor, whereas hyperactive variants of *YDA* suppress formation of the embryo often to the extent that all daughters of the zygote appear to be suspensor-like. On the basis of these findings, we have proposed that the *YDA* MAPK cascade acts like a molecular switch that promotes extraembryonic or suspensor fate.

A mechanistic understanding of the YDA pathway will require an inventory of its components. Using various approaches, five other genes with a likely function in *YDA*-dependent signaling have been identified. Work in the lab of S. Zhang (University of Columbia, Missouri) has implicated *MPK3* and *MPK6* as redundant MAPKs downstream from *YDA* in postembryonic development. In collaboration, we have confirmed that the same two MAPKs also function in the embryo. An ongoing, systematic survey of Arabidopsis MAPKK genes in our lab has, so far, implicated *MKK9*, *MKK4*, and *MKK5*: Expression of hyperactive variants in the embryo has a similar, although often weaker, effect as expression of hyperactive *YDA* variants. Thus, the *YDA* MAPK cascade appears to consist of *YDA*, *MKK9/4/5*, and *MPK3/6*. This MAPK cascade is required throughout the life cycle, regulating, among other processes, the size of the stem cell population in the shoot apical meristem and the formation of guard cells in the leaf epidermis.

In contrast, two components of the YDA pathway we originally identified by virtue of their *yda*-like mutant phenotypes in the embryo are not required after germination. The *GRD* gene likely executes suspensor-specific gene transcription in response to the *YDA* MAPK cascade. *GRD* encodes a protein of the RWP-RK family, a small group of predicted transcription factors that have only been found in green algae and higher plants. The only members of this family with an assigned function both affect developmental fate decisions: *minus dominance* (*mid*) from the unicellular alga *Chlamydomonas* dominantly determines the mating type of gametes, whereas *nodule inception* (*nin*) from the legume *Lotus japonicus* promotes the colonization of roots with nitrogen-fixing symbiotic bacteria in response to Nod-factor signaling. The *GRD* gene product contains two possible MAPK phosphorylation sites, one of which has a high score with different prediction algorithms and is conserved in the rice homolog of *GRD*. We have constructed variants of *GRD* in which one or both of these sites have been mutated such that they cannot be phosphorylated any more or such that they are mimicking the effects of constitutive phosphorylation. These variants are being tested in mutant plants.

Activation of the YDA MAPK cascade appears to require the *SSP* gene. *SSP* encodes a member of the receptor-like protein kinase family. In animals, these proteins operate in association with cell surface receptors, such as the interferon-1 receptor complex. We have found that SSP protein is myristoylated as well as palmitoylated at its amino terminus and that these fatty acid modifications effectively anchor the protein in the plasma membrane. Both modifications are essential for normal function: Mutation of the modified glycine or

cysteine residue carrying the modifications cause mislocalization of the SSP protein to the cytoplasm where it remains inactive. Although SSP has been classified as a protein kinase, key positions of the catalytic domain are not conserved, and SSP variants that harbor transition-state mutations in the ATP-binding pocket fully complement the phenotype of mutant embryos. This would suggest that SSP is a "dead" kinase. On the other hand, a small carboxy-terminal TRP domain of SSP that likely mediates protein–protein interactions is absolutely essential: Even deletions of a few amino acids create completely inactive variants. Taken together, our findings suggest that SSP functions as an adapter protein at the plasma membrane.

What is the role of SSP in the *YDA*-dependent signaling event? Unexpectedly, we found that SSP links activation of the *YDA* MAPK cascade to fertilization. Mutations in SSP show a very unusual parent-of-origin effect: The phenotype of the zygote and embryo is completely determined by the genotype of the pollen, i.e., wild-type egg cells fertilized with mutant pollen all develop as mutants, whereas mutant egg cells fertilized with wild-type pollen all develop as wild type. This paternal or male gametophytic effect appears to be mediated by a tight control of SSP production in the context of double fertilization. SSP transcripts are expressed specifically in mature or germinating pollen and, as judged from in situ hybridization, accumulate in the two sperm cells. However, we have been unable to detect the SSP protein in pollen, implying that translation of the mRNA is blocked. SSP protein can be visualized after fertilization, where it outlines the zygote as well as the micropylar endosperm, the other product of the double fertilization event, This would suggest that SSP transcripts are contributed to the zygote and endosperm by the sperm cells. Consistent with this view, in situ hybridization to sections of immature seed reveal a weak but reproducible signal of SSP mRNA in the zygote and the surrounding endosperm.

Why is the production of SSP controlled in such a precise fashion? Forced expression studies demonstrate that ectopic production of SSP is sufficient to trigger *YDA*-dependent signaling in the cells of the leaf epidermis, implying that *SSP* can act as a signal. Taken together, our work suggests a novel and truly unconventional mechanism for regulating MAPK signaling in plant embryos and immediately raises a number of questions surrounding this event: How is *SSP* mechanistically connected to activation of the *YDA* MAPK cascade? When after fertilization does the *YDA* pathway become active and how long does it stay active? Our current research focuses on addressing these issues.

A GATA Factor Positioning the Proembryo Boundary

W. Lukowitz, T. Nawy

The embryonic root of *Arabidopsis* develops at the boundary between the two cell types established with the asymmetric division of the zygote: the suspensor and proembryo. Root initiation is triggered by an inductive signal from the lower-tier cells of the proembryo to the uppermost suspensor cell and is dependent on the plant hormone auxin.

Mutations in the GATA-type transcription factor *HAN* eliminate all anatomical hallmarks associated with root formation in the early embryo. However, about 20% of all *han* mutants eventually recover and, after a lag period, form relatively normal seedlings. This unique feature of *han* is in striking contrast to mutations in the auxin signaling pathway, which typically result in rootless seedlings and affect development throughout the life cycle. Thus, *HAN* is not required for root formation per se but specifically for initiating a root in the early embryo.

An analysis of *han* mutants with a panel of molecular cell-fate markers revealed that the expression of root-specific genes is not absent in the mutants but rather shifted toward the center of the proembryo. For example, a *WOX5* reporter gene, normally expressed in the uppermost suspensor cells and subsequently the center of the incipient root, is found in a broad stripe across the center of the proembryo. These observations suggest that *han* mutations change the coordinates of the fate map, shifting it toward the apex.

How can this global effect on the fate map be rationalized? The only known pathway with a global effect on embryonic development is auxin signaling: Does *HAN* influence auxin perception? Mutations in *ha*n suppress the root-less embryo phenotype caused by loss of the auxin response factor gene *MP:* In contrast to *mp* single mutants, *mp han* double-mutant embryos do express molecular markers for the incipient root and *mp han* seedlings eventually form a normal root. Our initial conclusion from this finding was that *HAN* inhibits select auxin responses, perhaps in direct antagonism to *MP*. However, further tests did not recover any support for this view. Instead, the genetic interaction between *han* and *mp* is likely due to the peculiar way in which *han* embryos form an incipient root: They do so at a time different from wild type and from founder cells that apparently are more tolerant to the loss of *MP*.

Rather than acting on auxin perception or responses, *HAN* may alternatively be involved in organizing auxin

transport and distribution. Auxin flux in the early embryo is dynamic and mediated by two groups of PIN efflux transporters. After division of the zygote, PIN7 accumulates in the apical membranes of the basal daughter and subsequently suspensor cells, pumping auxin upward. Modest levels of auxin presumably accumulate in the apical cell and are required for sustaining its development to the proembryo. Once the proembryo has about 32 cells, the direction of auxin transport is reversed through an unknown mechanism, and apical-to-basal auxin flux, one of the defining features of plants, becomes established. This reversal of auxin flux coincides with the accumulation of PIN1 and related transporters in the basal membranes of the proembryo cells and causes an accumulation of auxin in the uppermost suspensor cell. It is the auxin maximum in the uppermost suspensor cell that has a key role in triggering root initiation across the suspensor-proembryo boundary.

HAN has a surprising and profound impact on auxin flux in the early embryo, regulating the spatial expression of both PIN7 and PIN1. PIN7 expression, normally confined to the suspensor, expands into the proembryo of *han* mutants, resulting in the accumulation of PIN7 protein in the apical membrane of the lower tier cells (Fig. 1). In a mirror image, PIN1 expression becomes restricted to the cells of the upper tier. These findings would suggest that the auxin maximum is shifted apically in *han* mutants, a prediction that is confirmed by the analysis a synthetic auxin-responsive reporter: A new boundary between cells showing strong auxin responses and cells showing no detectable response forms in the center of *han* proembryos, resulting in root initiation at this ectopic position. Taken together, *HAN* appears to be required for positioning an instructive boundary that normally forms between the suspensor and the proembryo. It remains to be investigated whether this boundary is specified through auxin, i.e., whether the phenotype of *han* embryos can be explained by the effect of han mutations on auxin transport.

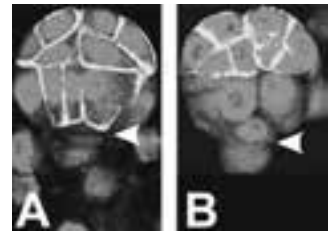

FIGURE 1 Apical shift of PIN1 expression in *han* embryos. The PIN1 auxin efflux carrier accumulates preferentially in the basal membranes of wild-type proembryos (*A*), but expression is limited to the upper tier cells in *han* mutants (*B*). Embryos at the 32-cell stages are shown; their nuclei are stained with 4′-6-diamidino-2-phenlyindole (DAPI) (*gray*), and the proembryo-suspensor boundary is marked with an arrowhead.

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PLANT DEVELOPMENTAL GENETICS AND FUNCTIONAL GENOMICS

R. Martienssen A.-Y. Chang, K. DePre, P. Ferreira, E. Gruntman, J.-J. Han, D. Irvine, A. Kloc, S. Locke, E. Nora, U. Ramu, R. Schwab, R. Shen, J. Simorowski, R.K. Slotkin, M. Tanurdzic, M.W. Vaughn, J. Xue, M. Zaratiegui

Transposon silencing, gene control, and stem cell function in plants, as well as heterochromatic silencing in yeast, provide useful models for regulation in higher organisms. In fission yeast, RNA interference (RNAi) of centromeric transcripts regulates histone modification, and we have found similar transcripts in *Arabidopsis*, where small RNA, DNA methylation, and chromatin remodeling regulate heterochromatin through transposons and repeats. We have found that these mechanisms function during chromosomal replication and have a role in epigenetic inheritance. Stem cell function and axis formation in *Arabidopsis* depends on *asymmetric leaves1* and on RNAi via spatially restricted microRNA (miRNA) and *trans*-acting small interfering RNA (siRNA). We continue to develop transposons and artificial miRNA as tools to probe genomic function.

During the past year, we were joined by graduate students Jong-Jin Han, An-yun Chang, Eyal Gruntman, and Undergraduate Research Program participant John Xue. We said goodbye to visiting student Elphege Nora, who returned to Paris, and sabbatical visitor Paulo Ferreira, who returned to the University of Rio de Janeiro.

RNAi Guides Histone Modification during the S Phase of Chromosomal Replication

A. Kloc, M. Zaratiegui, E. Nora

Heterochromatin remains condensed throughout the cell division cycle and silences genes nearby, but the mechanism by which heterochromatin is inherited has remained obscure. Heterochromatic silencing and histone H3 lysine-9 methylation (H3K9me2) depend, paradoxically, on heterochromatic transcription and RNAi. We have shown that heterochromatin protein 1 in fission yeast (Swi6) is lost via phosphorylation of H3 serine-10 (H3S10) during mitosis, allowing heterochromatic transcripts to transiently accumulate in S phase. Rapid processing of these transcripts into siRNA promotes restoration of H3K9me2 and Swi6 after replication when cohesin is recruited. RNAi in fission yeast is inhibited at high temperatures, providing a plausible mechanism for epigenetic phenomena that depend on replication and temperature, such as vernalization in plants and position-effect variegation in animals. These results explain how "silent" heterochromatin can be transcribed and lead to a model for epigenetic inheritance during replication (Fig. 1).

Heterochromatin, Histone Modification, and RNAi in Fission Yeast

M. Zaratiegui, D. Irvine, S. Locke, A.-Y. Chang, M.W. Vaughn
[in collaboration with Yang Shi, Harvard Medical School; Z. Cande, University of California, Berkeley]

We are applying a combination of forward and reverse genetics, genomics, and biochemistry to further explore the link between RNAi, histone modification, and heterochromatic silencing in the fission yeast *Schizosaccharomyces pombe*. In one example, the histone demethylase LSD1 represses and activates transcription by demethylating histone H3K4me2 and H3K9me2, respectively. Genetic ablation of the *S. pombe* homologs, *splsd1* and *splsd2*, resulted in slow growth and lethality, respectively, underscoring their physiological importance. Lsd1 and Lsd2 form a stable protein complex, which exhibits demethylase activity toward methylated H3K9 in vitro. Both proteins were associated with the heterochromatin boundary regions and euchromatic gene promoters. Loss of spLsd1 resulted in increased H3K9me accompanied by reduced euchromatic gene transcription and heterochromatin propagation. Removal of the H3K9 methylase Clr4 partially suppressed the slow-growth phenotype of *lsd1* deletions. Conversely, catalytically inactivating point mutations in the *lsd1* and *lsd2* genes partially mimicked the growth and heterochromatin propagation phenotypes. Taken together, these findings suggest the importance of both

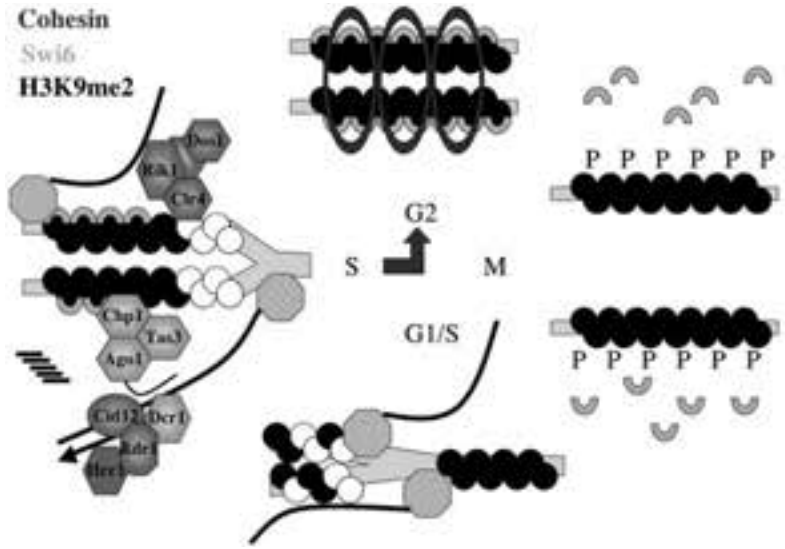

FIGURE 1 A model for cell cycle regulation of heterochromatic RNAi and histone modification. Cells in the G_2 phase of the cell cycle have high levels of H3K9me2 and Swi6 associated with heterochromatic repeats and retain cohesin. Phosphorylation of H3S10 in mitosis results in loss of Swi6 (heterochromatin protein 1) and transcription of the repeats in S phase. K9me2 levels fall during replication, but they are restored in S phase by RNAi of the transcripts, which spreads K9 methylation from unreplicated to replicated DNA, restoring levels of Swi6.

enzymatic and nonenzymatic roles of Lsd1 in regulating heterochromatin propagation and euchromatic transcription.

Transposable Elements and Epigenetic Control in Plants

R.K. Slotkin, M. Tanurdzic, E. Gruntman, R. Schwab, J. Xue, K. DePre, M.W. Vaughn [in collaboration with W. Thompson, North Carolina State University; R.W. Doerge, Purdue University]

Heterochromatin is composed of transposable elements (TE) and related repeats. Like TEs, heterochromatin silences genes located nearby and has a major role in epigenetic regulation of the genome. siRNA corresponding to heterochromatic sequences can be detected in plants, animals, and fission yeast, indicating that these sequences are transcribed. In plants, siRNA corresponding to different classes of TEs depends on the DNA methyltransferase MET1, the SWI/SNF ATPase, DDM1, or both, but not on the histone deacetylase SIL1. All three genes are required for silencing transposons in the absence of RNAi, but they depend on siRNA for resilencing in backcrosses. We are using gene-trap insertions within silent TEs of various classes, as well as developmental profiling of transcripts and siRNA, to determine the origin of TE silencing mechanisms in plants. In one example, plant cells grown in culture exhibit genetic and genomic instability. Using a tiling microarray, we have mapped the location and abundance of histone and DNA modifications in a rapidly dividing, dedifferentiated cell suspension culture of *Arabidopsis*. We have found dramatic changes in DNA hypomethylation and activation of specific TEs in culture. High-throughput sequencing of siRNA revealed that TEs activated in culture have reduced levels of a specific size class of siRNA. These results implicate RNAi and chromatin modification in the activation of TEs in undifferentiated cells.

Epigenetic Natural Variation and Polyploidy in *Arabidopsis thaliana*

M. Tanurdzic, M.W. Vaughn [in collaboration with L. Comai, University of California, Davis; R.W. Doerge, Purdue University; V. Colot, ENS, Paris, France]

Polyploidy in plants results in a variety of genetic and epigenetic changes in gene expression from generation to generation following the establishment of allopoly-

ploids. The mechanisms that underlie these changes are important in the short term (hybrid sterility) as well as in the long term (hybrid vigor), as polyploids are stabilized during evolution. We are using microarrays and Solexa sequencing to profile chromatin modifications and small RNA in synthetic allopolyploids from crosses between tetraploid *A. thaliana* and tetraploid *A. arenosa*. We profiled methylated DNA using tiling microarrays of *Arabidopsis* Chromosome 4 in two diploid ecotypes: Columbia and Landsberg. Repeated sequences and TEs, especially long terminal repeat retrotransposons, are densely methylated, but one third of the genes also have sparse methylation in their transcribed regions. Although TEs are almost always methylated, genic methylation is highly polymorphic, with half of all methylated genes being methylated in only one of the two ecotypes. A survey of loci in 96 *Arabidopsis* accessions revealed a similar degree of methylation polymorphism. Within-gene methylation is heritable but is lost at a high frequency in segregating F_2 families. Promoter methylation is rare, and gene expression is not generally affected by differences in DNA methylation. siRNAs are preferentially associated with methylated TEs, but not with methylated genes, indicating that most genic methylation is not guided by siRNA. This may account for the instability of gene methylation if occasional failure of maintenance methylation cannot be restored by other means.

Efficient Gene Silencing by Artificial miRNAs in *Arabidopsis*

R. Schwab [in collaboration with D. Weigel, Tuebingen; W.R. McCombie and G. Hannon, Cold Spring Harbor Laboratory]

RNAi is a powerful tool for gene discovery in plants, as well as for genetic modification. The latest RNAi vectors contain short hairpins that are modified precursors of miRNAs, a class of endogenous small RNAs. These produce a distinct small RNA sequence, which can be modified and optimized, and is often called artificial miRNA (amiRNA). A systematic approach is currently being used to generate thousands of hairpins to silence the majority of transcripts encoded by the model plant *A. thaliana*. Numerous successful applications of amiRNAs in *A. thaliana* have been reported, but about 20% of these amiRNAs did not trigger detectable gene silencing. We are investigating the nature of unsuccessful gene silencing in *A. thaliana* by directly analyzing the effect of local secondary structures in target transcripts and also by an unbiased search for additional determinants.

Maize-targeted Mutagenesis: A Knockout Resource in Maize

P. Ferreira, J.-J. Han, M.W. Vaughn [in collaboration with W.R. McCombie, Cold Spring Harbor Laboratory]

The maize-targeted mutagenesis (MTM) population comprises seed and tissue from 44,000 maize plants in which *Mutator* transposons have been mobilized and then stabilized genetically. Tissue was harvested in 18 two-dimensional "grids" of 48 x 48 plants each, and genomic DNA was prepared from row and column pools that permit cross-referencing of any individual sample. Target sites flanking each *Mutator* insertion can be amplified en masse from these pools by a variety of polymerase chain reaction (PCR) methods. Sequencing using massively parallel Illumina (Solexa) technology followed by mapping back to the maize genome resulted in identification of a high proportion of the flanking sites, including control insertions previously recovered by conventional means. By first comparing nonintersecting row pools, and then row and column pools, it is possible to distinguish preexisting (parental) insertions from new germinal insertions at row-column intersections. We estimate that approximately 0.5 to 1 million independent new insertions of *Mutator* elements in the gene-rich portion of the genome can be identified with only a few hundred sequencing reactions.

Gene Trapping and Chromatin Charting in *Arabidopsis*

J. Simorowski, U. Ramu, R. Shen [in collaboration with D. Spector and W.E. McCombie, Cold Spring Harbor Laboratory; E. Lam, Rutgers University]

Our collection of *Arabidopsis* gene-trap and enhancer-trap transposon lines has grown to more than 40,000 individual insertions, more than half of which have been mapped to the genome by PCR and sequencing. The resulting knockouts, along with phenotypic and expression data, are made available to the public via TRAPPER (http://genetrap.cshl.edu/), an interactive database and ordering system. Reporter genes are silenced by position-effect variegation when they are located a few kilobases from the NOR (nucleolar organizer) and depend on heterochromatic RNAi for silencing. Similar insertions in centromeric satellite repeats are also silenced. We have expanded this system to include gene-trap transposons with *lacR*-GFP (green fluorescent protein) chromosome charting beacons that

enable chromosomal locations to be visualized in the nucleus. These can be used to visually track individual loci in living plants while simultaneously producing a genome-wide "transcription potential map" via a common luciferase expression cassette. A direct correlation among mobility constraint, subnuclear location, and gene activity was observed in some cases. We provide a resource for examining epigenetic control in living cells within chromatin regions at the kilobasepair level of resolution (http://charting. cshl.edu).

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Danielle Irvine

PLANT SIGNALING AND DEVELOPMENT

D. Jackson Y. Benitez, P. Bommert, D. Bouyer, K. Chen, S. DeBlasio, A. Gallavotti, R. Johnston, S. Kotkin, B.-H. Lee, A. Mohanty, A. San Roman, N. Satoh-Nagasawa, J. Wang, C. Whipple, Y. Yan, P. Yin, T. Zadrozny

Our major goal is to identify genes and signals that regulate plant growth and architecture. 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 work. In the last year, we have identified a new gene that controls how plant cells communicate with each other through small channels, called plasmodesmata. This gene is necessary for the cell-to-cell movement of an important signal that is contained in the KNOTTED1 homeodomain protein. KNOTTED1 is a transcription factor that is expressed in the plant stem cells and is necessary to keep these cells in an undifferentiated state. Plasmodesmata are tiny channels that link plant cells to their neighbors, and they are critical for plant growth because they allow the flow of nutrients and signals through growing tissues.

We also continue to identify maize genes with novel roles in development. In the past year, genes that control branching, stem cell proliferation, and leaf growth have been identified. One of these, *TILLERED1*, encodes a transcription factor that controls the growth of branches, thereby controlling the overall shape or architecture of the plant. This gene is particularly interesting because it responds to light and may provide a way in which the plant can control its growth in response to environmental conditions. Finally, we and colleagues have begun to assemble a collection of "fluorescent-protein"-tagged maize lines that will be an important 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.

Regulation of Phyllotaxy in Maize

R. Johnston, B.-H. Lee, T. Zadrozny

Leaves are positioned in ordered arrays along the stem, forming patterns that are characteristic for different plant species. This arrangement on the shoot axis is termed phyllotaxy. These patterns can be traced back to events in the shoot apical meristem (SAM)—the reservoir of stem cells at the shoot tip that is the site of leaf initiation. The aim of this project is to investigate the mechanisms that determine these patterns of leaf initiation.

The maize plant exhibits a very ordered pattern of growth, making it an excellent model for the study of phyllotaxy. Leaves of the main axis are initiated singly in an alternate pattern, generating a distichous phyllotaxy. *aberrant phyllotaxy 1* (*abph1*) mutants, in contrast, have an altered phyllotaxy—leaves are initated in pairs in a decussate pattern. Previous work by our lab has shown that *abph1* encodes a cytokinin-inducible type-A response regulator (type-A RR) that is involved in the negative regulation of cytokinin signaling, indicating a role for cytokinin in phyllotactic patterning.

The plant hormone auxin is also implicated in leaf initiation and phyllotaxy. Polar auxin transport is facilitated by the PIN1 protein. We found that PIN1 and auxin levels are reduced in *abph1* SAMs. To elucidate interactions between auxin and ABPH1, we are using transgenic lines expressing the PIN1 protein fused to a yellow fluorescent protein and ABPH1 fused to a red fluorescent protein. Confocal microscopy is being used to investigate the localization of these two proteins in the SAM. In addition, we are conducting experiments in which *abph1* embryos are cultured on auxin and the effects on meristem size and phyllotaxy are determined.

To determine if the regulation of phyllotaxy by type-A RRs is conserved in other species with different phyllotactic patterns, type-A RR function is also being investigated in the model dicot *Arabidopsis thaliana*. We have obtained *Arabidopsis* lines that contain an ethanol-inducible construct encoding an artificial microRNA targeting *ARR7* and *ARR15* (from Jan Lohmann, MPI, Tuebingen). These plants are being analyzed for alterations in phyllotaxy.

To further understand the regulation of maize phyllotaxy, we are characterizing a second mutant, *Abph2*. This mutation is dominant, and, unlike *abph1*, *Abph2* phyllotaxy is initially normal and changes to decussate at about leaf 5. This change is often preceded by the pro-

duction of a fused leaf. Analysis of embryos indicates that *Abph2* SAMs are considerably wider than nonmutant SAMs as early as 20 days after pollination. The identity of *Abph2* remains unknown, and efforts are under way to isolate this gene by positional cloning. The location of *Abph2* has been narrowed down to a region of chromosome 7 between the predicted genes AC195322.2_FG002 and AC201967.3_FG002. Because there is a gap in the sequence information available for this region of the maize genome, sequence similarity (synteny) with the rice genome is being used to further define the position of *Abph2*. Markers are being developed based on rice genes contained within this interval.

We are also conducting a mutagenesis screen to help identify additional alleles of *Abph2*. Pollen from homozygous *Abph2* plants was treated with ethylmethanesulfate (EMS), a mutagenic compound, and then used to pollinate nonmutant plants. Seeds obtained from these crosses are currently being screened. Most plants will have the dominant *Abph2* phenotype. However, in cases where mutation has disrupted the *Abph2* allele, the progeny should appear normal. It is hoped that sequencing of genes from such "knockout" plants will provide further clues as to the identity of *Abph2*. In addition, this approach may generate *abph2* loss-of-function alleles that will provide information about the normal role of *abph2* in plant growth and development and will help elucidate the mechanism by which it regulates maize stem cell function.

Investigating Cell-to-Cell Transport Using Trichomes for Genetic Screens

J. Wang, D. Bouyer, S. Choi

We performed genetic screens to identify regulators of intercellular protein transport in plants. Plants use specific gates, plasmodesmata, to exchange nutrients and macromolecules, such as proteins and RNA, between cells. In fact, plasmodesmata are used to coordinate developmental processes throughout the plant's lifetime. Although the existence of these channels has been known for a long time, little is known about their molecular composition and regulation. To characterize this transport mechanism, we made use of certain proteins that are mobile between cells. The mobility of these proteins can be monitored by an epidermal phenotype, the production of leaf hairs, called trichomes.

We performed a genetic screen to find factors that regulate the cell-to-cell transport of the KNOTTED1 (KN1) homeodomain protein, the first plant transcription factor found to move between plant cells. A fusion of the homeodomain of KN1 with the GLABROUS1 (GL1) protein was able to complement trichome formation when expressed in the non-trichome-producing subepidermal cells of a *gl1* mutant line. We showed that this rescue was dependent on the ability of the KN1-GL1 fusion protein to move into the epidermal cells. Four mutants have been identified in this screen, and we mapped each to a small region of the *Arabidopsis* genome. For two of the mutants, we amplified these regions and sequenced them using Solexa high throughput sequencing. In one of the regions, a single open reading frame is disrupted by a point mutation that causes an amino acid substitution. The encoded protein is predicted to have a role in protein folding and is also thought to be associated with membranes. Proteins need to undergo partial unfolding prior to passage through the plasmodesmal pore, so this protein could be a core constituent of these channels. Experiments to localize the protein are in progress. Analysis of any genes isolated using our screen should be informative as to the mechanism of intercellular transport of KN1 and other mobile plant signals.

We also performed a screen using a negative regulator of trichome development that leads to reduction of trichomes when expressed from the subepidermis and screened for mutants that revert to normal trichome production, which would be expected if the negative regulator is no longer transported to the epidermis. So far, we isolated six mutants from this screen, which will be analyzed in more detail in the future.

On the other side, we made use of a positive trichome regulator, *TRANSPARENT TESTA GLABRA 1* (*TTG1*). *TTG1* is able to complement the *ttg1* mutant when expressed from the subepidermis. Thus, the ability of TTG1 to move between cells can be used to screen for transport-deficient mutants, which should show a reduction in trichomes. We isolated 15 mutants from this screen and they are now being characterized. *TTG1* is also interesting because it shows a functional conservation as well as diversification during evolution. The regulation of anthocyanin pigment production by *TTG* is conserved throughout all plant species analyzed so far. However, its involvement in trichome development is not shared between different plants. Interestingly, the feature of cell-to-cell mobility correlates with the involvement of *TTG1* in trichome initiation, a process that requires cell-to-cell communication. The mobility domain has been narrowed down to about 60 amino acids that either confer or prevent the mobility of the protein. This offers the possibility of analyzing an evolutionary aspect of protein transport and its regulation.

Thioredoxin-dependent Regulation of Intercellular Trafficking

Y. Benitez, M. Cilia, A. San Roman, M. Gonzalez, S. Hearn

Plasmodesmata are cell-wall channels connecting plant cells. Through them, metabolites, viral and endogenous proteins, and mRNA are transported. This communication is environmentally and developmentally controlled, but the molecular mechanism underlying this regulation is not well understood. To address this question, *A. thaliana* plants expressing the green fluorescent protein (GFP) driven by a phloem-specific promoter were mutagenized. In these plants, free GFP is able to diffuse, being transported through PD out of the phloem. We screened for mutants blocked in this transport. This genetic approach led to the identification of five mutants potentially involved in plasmodesmata regulation. These mutants were affected in GFP unloading from the phloem to the root meristem. Therefore, we named them gat (pronounced "gate") for "GFP arrested trafficking."

In *gat1*, GFP was not transported from the phloem in either the root or the shoot. Transmission electron microscopy showed that plasmodesmata channels may be branched and obstructed in the mutant, which might be the cause of reduced intercellular communication. *gat1* plants do not develop meristems, and they die at an early seedling stage. This gene encodes a thioredoxin protein that is localized in plastids of shoot and root meristems and developing tissues (Fig. 1). Thioredoxins have a role in the redox regulation of metabolic and stress-related processes including sugar storage, photosynthesis, and defense. High levels of reactive oxygen species (ROS) were found in *gat1* root meristems, and the mutant trafficking defects were phenocopied in wild-type plants germinated in the presence of oxidants. These data suggest that GAT1 is involved in the regulation of symplastic transport by a ROS-dependent pathway. Increased callose deposition and decreased starch content in the root meristems of these mutants were also observed. Callose involvement in the constriction of plasmodesmata channels has been well documented, and we speculate that *gat1* plasmodesmata are also obstructed by callose. Therefore, like GFP, sugars produced in the leaves during photosynthesis and translocated in the phloem are not unloaded in the root meristem. A direct supply of sucrose is necessary to maintain the high growth rate characteristic of meristems, and the excess is stored as reserve in the form of starch granules. The arrested meristem growth and the lack of starch granules in *gat1* mutants are therefore a consequence of the observed restricted trafficking phenotype. Agreeing with this hypothesis, *gat1* plants are partially complemented when sucrose is provided at germination, and plants can be maintained by this treatment. Moreover, the ectopic expression of GAT1 in mature leaves of wild-type plants led to an increase accumulation of starch, delayed senescence, and a delayed flowering. These plants also showed increased symplastic transport capability as reported by the transport of GFP from a primary target site in biolistic experiments. These results indicate that GAT1 is both necessary and sufficient to regulate symplastic communication and carbohydrate metabolism, which are both important in maintaining stem cell functions.

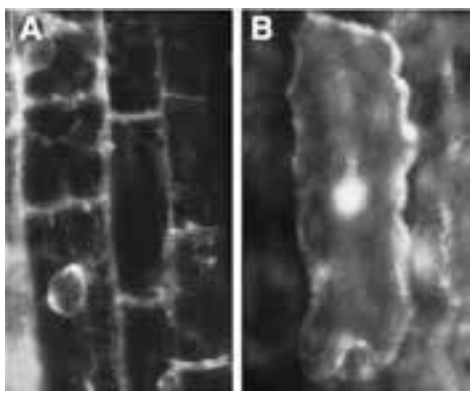

FIGURE 1 Differential subcellular localization of a cytokinin response regulator. Images show the localization of an ARR7-YFP fusion in root cells (*A*), where it is predominantly cytoplasmic, and in the leaf (*B*), where the fusion protein enters the nucleus.

Hormone Signaling in Maize

S. DeBlasio, T. Zadrozny, A. Mohanty, Y. Yan [in collaboration with A. Chan, The J. Craig Venter Institute; A. Sylvester, University of Wyoming]

Similar to animal systems, hormones also have a key role in the proper growth and development of plants. The formation and patterning of lateral organs (i.e., roots, leaves, and flowers) in vital crop species such as maize depend on the hormonally induced changes in gene transcription that occur within meristematic stem cells in response to cues from the environment. How well these organs develop under normal and stressful conditions often has major effects on important agronomic processes including crop yield and nutrient content. One such class of plant hormones that acts to mediate organ morophogenesis is the cytokinins.

In conjunction with another phytohormone, auxin, cytokinins act to maintain the population of stem cells

within the meristem as well as to promote their differentiation by interacting with histidine kinase (HKs) receptors located on the plasma membrane. This initiates a phosphorelay cascade within the cell similar to the two-component signaling transduction pathway first discovered in bacteria. Binding of the hormone to the extracellular surface of the HK induces conformational changes across the membrane, which causes the cytosolic kinase domain to transfer its phosphate group to a histidine phosphotransfer (HP) protein. Once phosphorylated, the HP protein shuttles from the cytoplasm to the nucleus and passes on the phosphate to a specific group of transcription factors known as B-type response regulators (RRs). Activation of the B-type RRs leads to changes in gene expression of proteins involved in various developmental pathways including cell elongation, chloroplast differentiation, and cell division. B-type RRs also induce transcription of A-type RRs, which go on to negatively inhibit the cytokinin phosphorelay cascade.

Although a lot has been learned from studying how the cytokinin pathway works in the model plant system *A. thaliana*, our lab is interested in understanding how the hormone regulates organ formation in maize, a monocot plant species that displays a pattern of organ development different from that of *Arabidopsis*. With the recent advent of more efficient transformation techniques and the near completion of the Maize Genome Sequencing project, we have focused on obtaining transgenic plants that natively express fluorescently tagged versions of the maize HPs, HKs, RRs, cytokinin oxidases, and putative cytokinin-responsive proteins such as the cell cycle control protein cyclin D to determine their (1) tissue specificity, (2) subcellular localization, and (3) patterning during development. Specifically, we are interested in knowing how and if regulation of their localization during development differs from what has been seen for other flowering plants, as well as to study their expression patterns in maize organs not found in *Arabidopsis*, such as the male reproductive structure, or tassel.

To date, our lab has successfully created stable maize lines expressing at least one member of the cytokinin-specific protein families mentioned above and are in the process of characterizing their localization patterns in multiple organs and cell types using confocal image analysis.Interestingly, preliminary results suggest that the subcellular localization of some these proteins may differ between organ types. For example, in T1 transgenic maize seedlings expressing ARR7-YFP (an A-type RR), the protein is located mainly in the cytoplasm and around the nucleus of root cells (Fig. 1A). However, in leaf epidermal cells, a greater concentration of ARR7-YFP was observed within the nucleus (Fig. 1B).

We have also generated a series of marker lines that highlight different subcellular compartments, including tonoplasts, nuclei, peroxisomes, microtubles, plastids, and various other structures that are made available to the scientific community. We plan to use these lines to analyze the changes that occur within the cell during differentiation and development.

Inflorescence Meristem Size Regulation in Maize

P. Bommert, P. Yin, J. Wang

The shoot apical meristem has a remarkable ability to regulate its size during development, by balancing stem cell proliferation with the incorporation of daughter cells into primordia. We are particularly interested in studying this process in maize, where a large number of meristem proliferation or "fasciated" mutants are available.

We cloned the *FASCIATED EAR2* (*FEA2*) gene and found that it encodes a leucine-rich repeat (LRR) receptor-like protein, orthologous to *Arabidopsis CLAVATA2*. We also isolated the *THICK TASSEL DWARF1* (*TD1*) gene as a homolog of the LRR kinase CLV1. In *Arabidopsis*, these proteins form a receptor complex that is activated by the CLV3 ligand and repress the stem-cell-promoting transcription factor WUSCHEL. Our genetic analysis of *fea2/td1* double mutants, however, suggests that the simple CLV1-CLV2 coreceptor model is more complex in maize, since double mutants are much more severe than either single mutant alone. To address the molecular nature of these effects, we are characterizing the FEA2 receptor complex using immunoprecipitation approaches. We are currently using a transgenic 35S::FEA2-GFP line in combination with anti-GFP antisera to isolate the FEA2 receptor complex. Our first results led to the identification of an uncharacterized maize protein that has homology with an LRR receptor-interacting protein from rice. This protein becomes phosphorylated after receptor activation, suggesting it may act downstream from the FEA2 receptor.

In addition to our biochemical approach, we are also pursuing a genetic approach to isolate the *compact plant2* (*ct2*) mutation. *ct2* mutants develop abnormally enlarged inflorescence meristems, indicating that this gene also affects meristem size regulation. Using approximately 900 *ct2* F_2 mutants, we narrowed down the chromosomal location of the gene to an interval of 250 kb on chromosome 1 covered by two BAC (bacterial artificial chromosome) clones representing eight genes. Subsequent sequencing of these genes in the *ct2-Muszynski* allele led

to the identification of a transposon insertion in a lipoxygenase-encoding gene. Lipoxygenases are enzymes involved in jasmonate biosynthesis. Jasmonates are phytohormones and are known to regulate plant growth and development. Cloning of the *ct2* gene therefore provides a possible direct link between jasmonate synthesis and/or signaling and meristem size regulation. To further substantiate this, we are currently screening for additional *ct2* alleles by a targeted EMS screen. Interestingly, our analysis of *ct2/td1* double mutants suggests that *ct2* identifies a pathway for regulation of meristem size that operates independently of the *CLAVATA* pathway.

Tassel sheath and *Tillered* Genes Regulate Diverse Aspects of Plant Architecture

C. Whipple, S. Kotkin, K. Chen

Aerial plant development can be interpreted as a reiteration of three basic units: the leaf, stem, and an axillary meristem that will itself grow out and reiterate this pattern. Together, these units constitute a phytomer that is repeated throughout plant growth and development. It is by modification of phytomer architecture that changes in morphology occur. Distinct phytomer morphologies characterize transitions in the plant life phase (e.g., vegetative to reproductive), as well as species differences. Although there is a lot known about the development of individual phytomer components, little attention has been given to how phytomer growth is modified either throughout the life cycle of a single plant or through evolution. We have been investigating two maize mutants that modify phytomer development to affect plant morphology.

An interesting phytomer modification that occurs in many plant species is the suppression of leaf development after the transition to reproductive development. For example, in most grass species, inflorescence leaves, also called bracts, fail to grow, although a rudimentary bract primordium does initiate. To understand the regulation of bract suppression in grasses, we have begun an investigation of maize mutants that fail to suppress bract development, known as *tassel sheath* (*tsh*) (Fig. 2). We have identified four different genes—*tsh1*, *tsh2*, *tsh3*, and *tsh4*—that have this phenotype. With assistance from Sean Kotkin (undergraduate) and Kay Chen (high school student), we have mapped several of these genes. The *tsh1* and *tsh4* mutants have been cloned, and both are expressed in the cells of the suppressed bract. Our preliminary expression analysis indicates that *Tsh4* regulates *Tsh1* and is thus upstream in a common pathway of bract suppression. Currently, we are investigating *Tsh1* in other cereal grasses (rice and barley) where this gene appears to have a conserved function. Ultimately, we hope to understand the entire genetic pathway that regulates leaf outgrowth, which could then be engineered to modify plant architecture for agronomic purposes.

FIGURE 2 Development of the *tasselsheath1* mutant of maize. Wild-type maize inflorescences suppress the growth of leaves (*left*). However, in the *tasselsheath* mutants (e.g., *tsh1*), this growth suppression is lost, and leaves grow out to subtend the inflorescence branches and the spikelet structures (*arrows, right*).

Another phytomer modification that varies in response to environmental cues is the control of axillary meristem outgrowth. In the grasses, axillary meristems initiate several leaf primordia and then stop development, creating a dormant bud. Subsequent growth of this bud depends on a combination of internal and external cues. When these buds are at the base of the plant, they can grow out to generate a new stalk, also called a tiller. Tiller growth is regulated by light signals, and so grasses produce fewer tillers in a shaded environment, such as when they are densely planted. We have cloned a maize mutant *tillered* (*till*) that produces extra tillers and fails to suppress axillary bud outgrowth. The *Till* gene is specifically expressed in the young leaves initiated by axillary buds. A collaboration with researchers at Cornell University has shown that expression of *Till* is regulated by light quality. An understanding of tiller growth has important implications for increased biomass production that will be necessary as we transition from fossil fuels to biofuels.

Characterization of the Function of the *RAMOSA3* Gene in Maize

N. Satoh-Nagasawa, T. Bourett, A. Mohanty, Y. Yang [in collaboration with R. Feil and J. Lunn, Max-Planck Institute of Molecular Plant Physiology, Golm; N. Nagasawa and H. Sakai, Pioneer Hi-Bred, Delaware]

To elucidate the genetic and molecular mechanisms of inflorescence branching, a major yield trait in crops, we

are analyzing the *ramosa3* (*ra3*) mutant of maize. By observing single- and double-mutant phenotypes, we found that *RA3* functions upstream of another *RA* gene (*RA1*) to establish the correct identity and determinacy of axillary meristems in maize inflorescences. *RA3* was cloned and found to encode a functional trehalose-6-phosphate phosphatase (TPP). TPP is an enzyme that catalyzes the metabolic conversion of trehalose-6-phosphate (T6P) to trehalose. We also found that *RA3* is expressed at the base of axillary meristems in young maize inflorescences.

From these studies, we developed two possible hypotheses for the molecular mechanism of *RA3* function. First, T6P or trehalose may act as a mobile sugar signal during axillary meristem development in maize inflorescences. Second, the RA3 protein itself may have a regulatory function to control the expression of other genes important for the determinacy of axillary meristems.

To determine which of these hypotheses are correct, we started several experiments. We investigated RA3 protein localization by fluorescent protein fusions and immunolocalization. RA3 protein was localized in the same domain as its mRNA, ruling out the possibility that it acts as a mobile protein signal. Transient bombardment assays of a RA3-YFP fusion protein showed that it was localized in cytosol and nuclei. This localization of RA3 protein has been confirmed by immunolocalization, and its nuclear localization supports the hypothesis that it may have a regulatory function. We have also measured the concentration of trehalose and T6P in young inflorescences of wild-type and *ra3* mutants and found no differences, suggesting that the sugar signal hypothesis may not be correct. Several additional experiments using transgenic plants are also in progress. We tested the influence of reducing the concentration of trehalose in axillary meristems by expressing bacterial trehalase in the meristems. Trehalase is an enzyme that degrades trehalose. If trehalose acts as a signal for morphogenesis of axillary meristems, then plants expressing trehalase should phenocopy *ra3* mutants. We have also made catalytically inactive RA3 alleles by point mutations in the enzyme active site. The mutated RA3 protein should complement *ra3* mutants if this protein has a role other than metabolism of trehalose. We found weak complementation in a preliminary experiment. These results lead us to think that the RA3 protein may have a regulatory function in addition to or instead of its enzymatic function in vitro.

While we examine *RA3* function in maize, we are also interested the interaction of *RA3* with other genes in maize and in the function of *RA3*-like genes in other plants. We have started to map *ra3* suppressors in maize and are isolating mutants in *Arabidopsis* in order to define the function of these genes in a dicot species that has very different inflorescence development. With these experiments, we hope to characterize the mechanism by which *RA3* regulates inflorescence stem cell fate and obtain a better functional insight of inflorescence development in maize.

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PLANT DEVELOPMENTAL GENETICS

M. Timmermans D. Chitwood, M. Declerck, C. Fernandez-Marco, M. Guo, C. Hu, M. Lodha, F. Nogueira, A. Sarkar

Development in higher plants is a continuous process as organs emerge throughout the entire plant life cycle, which for some plants extends over hundreds of years. The growing tip of a plant, referred to as shoot apical meristem (SAM), contains a population of stem cells that divide to maintain the SAM and to generate daughter cells from which lateral organs, such as leaves and flowers, arise. The research in our lab aims to understand the molecular mechanisms that distinguish indeterminate stem cells from their differentiating derivatives. In addition, we are studying the role of stem-cell-derived signals in the patterning of lateral organs. Observations from our lab indicate that microRNAs (miRNAs) are among these meristem-derived signals. Moreover, stem cells produce signals required for the establishment of determinacy. This process involves an epigenetic silencing mechanism, indicating that the switch from stem cell to differentiated cell is encoded not simply in DNA, but also by proteins associated with DNA.

Adaxial/Abaxial Patterning of Lateral Organs in Maize

F. Nogueira, D. Chitwood [in collaboration with M. Scanlon, Cornell University, Ithaca, New York; K. Ohtsu and P. Schnable, Iowa State University, Ames]

Outgrowth and patterning of lateral organs in plants depend on the specification of adaxial-abaxial (dorsoventral) polarity. We have shown that this asymmetry is generated by a novel patterning mechanism involving two distinct small RNAs. The 21-nucleotide miRNA, miR166, delineates the abaxial/lower surface of the leaf by restricting the expression domain of class III homeodomain leucine zipper (HD-ZIPIII) transcription factors that specify adaxial/upper fate. The abaxial-specific expression of miR166 is generated by a second small RNA, the *trans*-acting short interfering RNA tasiR-ARF, which accumulates on the adaxial side of incipient and developing leaves. Our findings indicate that organ polarity in leaves is ultimately specified by regulating the spatial accumulation and relative efficacy of tasiR-ARF and miR166. Despite our extensive knowledge regarding the biogenesis and action of small RNAs, little is known about the regulatory mechanisms that exist to establish the correct pattern and levels of small RNA activity.

Small RNAs are processed from long precursor transcripts, and plant genomes typically contain numerous precursor genes for the biogenesis of each particular small RNA. For instance, the maize genome contains at least nine *mir166* loci, suggesting that the miR166 accumulation pattern may result in part from the differential regulation of individual *mir166* family members. Detection of these miRNA precursors by in situ hybridization has been unsuccessful. We therefore used laser capture microdissection (LCM) in combination with reverse transcriptase–polymerase chain reaction (RT-PCR) to analyze the expression profiles of *mir166* genes in specific domains of the SAM and young leaves. Although some *mir166* family members displayed overlapping expression profiles, most *mir166* genes exhibited unique tissue and cell-type-specific expression patterns. One of the tissue samples tested included cells from the incipient leaf. Four *mir166* family members are expressed in that domain and are thus important candidates for establishing adaxial-abaxial leaf polarity. Interestingly, the *mir166a* precursor was found to be expressed in the stem cells at the tip of the SAM. This result was surprising, because these cells do not accumulate mature miR166 and in fact express the *hd-zipIII* targets at high levels. This suggests that miR166 accumulation in plant stem cells is regulated in part at the level of biogenesis or stability. In animals, miRNA activity in stem cells is similarly regulated at the level of biogenesis. How miRNA activity is controlled in stem cells and whether such control is unique to stem cells are questions for future research.

As mentioned above, adaxial-abaxial patterning in maize also depends on the activity the tasiRNA, tasiR-ARF, whose biogenesis from *tas* precursor transcripts is initiated by an miR390 cleavage step. LCM separating cells from the epidermal (L1) and subepidermal layers (L2) of the SAM shows that *tas* transcripts accumulate in both L1 and L2 layers. In contrast, the precursors of

miR390 are detected only in the L1 layer of the SAM. Using in situ hybridization, we have detected the accumulation of mature miR390 in the adaxial domain of the incipient leaf. More importantly, we found that miR390 accumulates in both the L1 and L2 layers of the incipient leaf. Our observations are consistent with a scenario in which miR390 is produced exclusively in the L1 layer but moves into the underlying cell layers. This presents the possibility that miRNAs may move over a few cell lengths from specific cell types, such as the epidermal layer, to act as developmental signals during pattern formation. We are currently also testing this possibility in *Arabidopsis*.

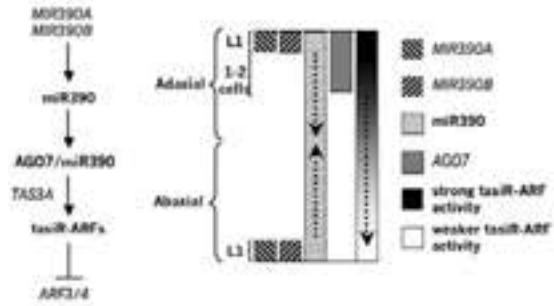

FIGURE 1 Summary of localization patterns of different tasiR-ARF biogenesis pathway members and a model of small RNA mobility (*dotted arrows*) in leaves. miR390 accumulates noncell autonomously throughout the leaf, outside of the L1 epidermis in which its precursors localize. *AGO7*, which is required for miR390 activity and thus the biogenesis of tasiR-ARFs, is only present in the adaxial-most cells of the leaf. The site of strongest tasiR-ARF activity on the adaxial side of leaves corresponds to the source of tasiR-ARF biogenesis; however, weaker tasiR-ARF activity that reflects mobility is found on the abaxial side of leaves.

Mobility of Small RNAs during Adaxial-Abaxial Patterning of the Arabidopsis Leaf

D. Chitwood, F. Nogueira [in collaboration with T. Montgomery, M. Howell, and J. Carrington, Oregon State University, Corvallis]

The importance of the contribution of miRNAs to development in multicellular organisms is well-established. However, the exact mechanisms by which these molecules contribute to development and possibly patterning remain understudied. A recurring theme in pattern formation is the interpretation of relative distance from a point source through the activity of a mobile positional signal. Our localization studies in maize suggest a noncell-autonomous component of the tasiR-ARF pathway, via miR390 mobility that establishes adaxial-abaxial polarity.

To more easily study the mechanisms by which such noncell autonomy occurs, we are continuing our studies in *Arabidopsis*. As in maize, precursors of miR390, as detected by in situ hybridization, accumulate exclusively in the L1 epidermal layer. This contrasts with the localization of mature miR390, which is detected throughout the leaf. At first glance, the localization of miR390 might suggest that tasiR-ARFs are produced throughout the leaf. However, miR390 requires ARGONAUTE7/ZIPPY for activity. *AGO7* reporter constructs indicate that *AGO7* is expressed in the adaxial/dorsal-most cells of leaves, limiting the activity of miR390 and thus tasiR-ARF biogenesis to these regions. Such a scenario is consistent with our localization of tasiR-ARF activity using transgenic sensor constructs, which show very strong tasiR-ARF activity on the adaxial side of leaves. However, weaker tasiR-ARF activity was also detected on the abaxial/ventral side of leaves outside the domain of *AGO7* expression and tasiR-ARF biogenesis. Consistent with these tasiR-ARF sensor results, in situ hybridization showed that tasiR-ARF accumulates in a graded pattern that dissipates from the adaxial to the abaxial side of leaves and may reflect mobility of tasiR-ARF from their source of biogenesis to the abaxial side of leaves.

Our model of the contribution of the tasiR-ARF pathway to leaf polarity through small RNA mobility therefore involves the movement of two different small RNAs: (1) miR390 moves from the epidermis into subepidermal layers and (2) tasiR-ARFs move away from the adaxial *AGO7*-restricted domain of miR390 activity, where they act most strongly, to the abaxial side of leaves, where they act weakly (Fig. 1). That movement of miR390 and tasiR-ARFs is essential for their patterning activity in leaves sets a precedent for the capacity of small RNAs to act as mobile positional signals and possibly even as morphogens in development.

Adaxial-Abaxial Patterning by AS1 and AS2

F. Nogueira, M. Declerck, M. Guo

In *Arabidopsis*, we are using various genetic and biochemical approaches to identify genes involved in the spatiotemporal regulation of the polarizing small RNAs. Several lines of evidence indicate that the DNA-binding proteins ASYMMETRIC LEAVES1 (AS1) and

ASYMMETRIC LEAVES2 (AS2) act together to regulate leaf morphology including adaxial-abaxial leaf polarity. We recently determined that AS1-AS2 bind as a complex to specific DNA sequence motifs in the promoters of target genes to regulate their expression. To begin to understand how AS1-AS2 contribute to organ polarity, we used chromatin immunoprecipitation (ChIP) to identify which members of the *MIR166* precursor family and components of the tasiR-ARF biogenesis pathway are direct targets of AS1-AS2 regulation. We found that *MIR166a*, *MIR166b*, *MIR166c*, and *TAS3a* are direct targets of AS1-AS2. To verify the ChIP results, we are using GUS reporter constructs and RT-PCR analysis to determine the effect of mutations in *as2* on the expression of these targets. For instance, we previously established that the full-length promoter of *MIR166a* drives expression only in the abaxial epidermal layer of young leaves. Using the *MIR166a::GUS* reporter, we have shown that *MIR166a* is adaxially misexpressed in an *as2* mutant background. Similarly, we have shown the transcript levels for *MIR166c* are increased in *as*2. Currently, we are also investigating the expression of a *MIR166c::GUS* reporter in wild-type and *as2* seedlings.

The abaxial determinants *ARF3* and *ARF4* are targets for tasiR-ARFs, and we have shown that transcript levels for both genes are up-regulated in *as2*. Considering that *TAS3a* is a direct target of AS1-AS2, these transcription factors may control *ARF3* and *ARF4* expression through regulation of tasiR-ARF accumulation. However, mutants defective in both the tasiR-ARF and the AS1/AS2 pathways show an enhanced leaf phenotype, suggesting that both pathways converge to repress *ARF3* and *ARF4* activity on the adaxial leaf surface. To test the interaction between the AS1/AS2 and tasiR-ARF pathways further, we generated *as2* plants containing a tasiR-ARF-insensitive allele of *ARF3* (*ARF3m*), which contains silent mutations that preclude the cleavage of the *ARF3* mRNA by tasiR-ARFs. The resulting mutants develop highly serrated abaxialized leaves with abaxialized ectopic leaf-like outgrowths in the margins. This is in contrast to the weak leaf polarity defects observed in double mutants defective for AS2 and tasiRNA biogenesis. Expression of *MIR166* precursors and its targets, and the expression of *ARF4* and components of the tasiR-ARF pathway are affected in the *as2 ARF3m* plants. The enhanced polarity defects resulting from expression of a tasiR-ARF-insensitive allele of *ARF3* in *as2* as opposed to losing tasiR-ARF activity in the *as2* background may reflect feedback regulation in the tasiRNA pathway. This hypothesis is currently being tested.

Establishment of Determinacy during Organ Development

M. Guo, M. Lohda, C. Hu

Indeterminacy within the SAM is specified in part by the *KNOX* homeobox genes. Down-regulation of *KNOX* expression is a key factor that distinguishes stem cells and their immediate derivatives in the SAM from lateral organ founder cells. Moreover, establishment of determinacy in developing organs requires the continued silencing of the *KNOX* genes. We have previously shown that this process involves the predicted DNA-binding proteins AS1 and AS2, as well as the chromatin-remodeling factor HIRA. On the basis of expression and genetic analyses, we proposed that these proteins are part of a novel cellular memory system that keeps *KNOX* genes in an "off" state during organogenesis, thus preventing differentiating cells from reverting into indeterminate stem cells. We have gained important new insights into the mechanism of *KNOX* gene silencing by the AS1 complex that confirm this hypothesis. ChIP experiments identified two sites in the promoter of the *KNOX* targets *BREVIPEDICELLUS* (*BP*) and *KNAT2* that mediate AS1-complex binding. Electrophoretic mobility-shift assays were subsequently used to precisely define the *cis* sequences required for binding of the AS1-AS2 complex to *BP*. Consistent with earlier genetic data, we found that AS1 requires AS2 for binding to the *KNOX* promoters and that this repressor complex binds to the regulatory motifs CWGTTD-KMKTTGAHW present at the two ChIP sites in the promoters of the *KNOX* targets. The significance of the two AS1-complex binding sites for stable *BP* silencing in leaves was demonstrated in vivo using various *BP promoter::GUS* reporter constructs. Deletion of either AS1-complex binding site resulted in misexpression of *BP* in wild-type leaves, indicating that both binding sites act nonredundantly to repress *BP* during organogenesis. This indicates that interaction between AS1-AS2 complexes at the two sites is required for stable repression of *BP* in leaves. Promoter deletion analysis further indicates that enhancer elements required for *BP* expression in the leaf are located between the AS1-AS2 complex binding sites. On the basis of these and earlier results, we present a model for AS1-AS2–mediated *KNOX* gene silencing that is conceptually similar to the action of an insulator. We propose that interaction between AS1-AS2 complexes creates a loop in the *KNOX* promoter and, likely through recruitment of HIRA, leads to the formation of a repressive chromatin state that maintains an enhancer region required for *KNOX* expression in leaves silenced throughout organogenesis (Fig. 2). We are

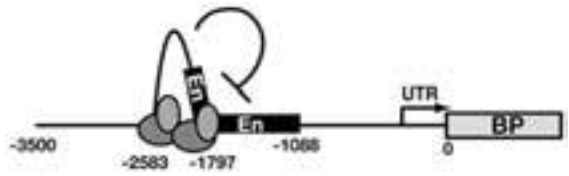

FIGURE 2 Model for AS1-AS2–mediated *KNOX* gene silencing in the leaf. The AS1-AS2 complex binds to regulatory motifs present at two sites in the promoters of its *KNOX* targets immediately upstream and surrounding an enhancer region required for expression in developing leaves. Interaction between the AS1 complexes is required for stable *KNOX* gene silencing, suggesting formation of a loop in the *KNOX* promoter that, likely through recruitment of HIRA, leads to formation of a stable repressive chromatin state that blocks enhancer activity throughout leaf development. (*Ovals*) AS1-AS2; (*rectangle*) leaf enhancers.

currently establishing chromatin conformation capture assays (3C) to detect the loop in vivo. We are also testing whether the AS1 complex is capable of forming a loop in the *BP* promoter using in vitro assays.

We have previously reported that AS1-AS2 interact with the chromatin-remodeling protein HIRA, which is involved both in heterochromatic gene silencing and in the epigenetic regulation of euchromatic genes. We are therefore studying the role of HIRA in *KNOX* repression during leaf development using genetic and biochemical approaches. As a first step, we are testing whether *KNOX* gene silencing is associated with changes in chromatin organization and/or DNA methylation. Preliminary data indicate that acetylation and methylation of specific histone residues on *BP* and *KNAT2* chromatin direct their activity in a tissue-specific manner. Additional observations also suggest a role for the Polycomb group of silencing proteins in the regulation of these *KNOX* genes. These data will eventually clarify the molecular mechanism that represses stem cell activity in differentiating lateral organs.

Regulation of Embryonic Patterning by HIRA

C. Fernandez-Marco

HIRA is a key developmental regulator in mammals and in plants. In mammals, loss of *HIRA* results in gastrulation and patterning defects that lead to early embryonic lethality. In plants, loss-of-function mutations in *HIRA* also lead to embryo lethality. However, reduced expression of *HIRA* causes leaf defects that resemble the defects observed in the *as1* and *as2* mutants and leads to misexpression of several *KNOX* genes. To further dissect the role of *HIRA* in stem cell homeostasis, we used the *Arabidopsis* TiLLing service to screen for ethylmethanesulfate (EMS)-induced mutations in the amino-terminal WD40-repeat domain and the carboxy-terminal region of *HIRA* that interacts with AS1. Thus far, we have obtained 12 independent *hira* alleles, which exhibit a range of developmental phenotypes, including defects in embryonic patterning, leaf development, and flowering. Perhaps the most interesting allele is *hira-serpent*, which contains a point mutation in the evolutionary conserved fifth WD40 repeat. The mutant phenotype is first apparent by the late globular stage of embryogenesis. Mutants are marked by abnormal patterns of cell division, both in the proembryo and in the extraembryonic suspensor. In particular, the protoderm cells of the proembryo show aberrant planes of cell division. Moreover, the hypophysis, which normally divides to form a lens-shaped cell that ultimately gives rise to the quiescent center of the root stem cell niche, divides abnormally in this *hira* mutant. To determine whether these morphological abnormalities are correlated with alterations in cell fate and patterning, we are examining the effects of this *hira* mutation on the expression of molecular markers in the developing embryo. Our preliminary results show that specification of the protoderm and of cell fates in the apical region is largely unaffected in this *hira* mutant. However, expression of the PIN7:GUS reporter, which normally marks the basal cell lineages of the preglobular embryo and the suspensor, was absent from the hypophysis and upper part of the suspensor in *hira-serpent*. Suspensor formation involves processes of central importance to embryogenesis such as establishment of apical-basal embryo polarity, early cell differentiation, and programmed cell death. The phytohormone auxin has a key role in these processes and in embryonic root formation. Crosses to marker lines that are expressed in the suspensor (IAA10:GFP and ARF13:GFP), in the hypophysis and lens-shaped cell (WOX5:GFP), or at auxin maxima (DR5:GFP) are in progress to test whether auxin signaling and/or cell-fate specification in the suspensor is perturbed. These data will shed new light into the fundamental principles of pattern formation in the plant embryo.

Global Expression Analysis of Meristem Function and Leaf Initiation

A. Sarkar [in collaboration with M. Scanlon, Cornell University, Ithaca, New York; P. Schnable, Iowa State University, Ames; B. Buckner and D. Janick-Buckner, Truman State University, Kirksville, Missouri]

Plant meristems comprise distinct histological and functional domains. For instance, the stem cells are located

at the most apical tip in the so-called central zone, whereas lateral organ founder cells are located on the flanks of the SAM. Traditional genetic analyses have demonstrated the importance of this meristematic organization for normal plant development and have led to the identification of some genes required for meristem function and lateral organ development. To identify novel and potentially redundant or essential genes that function in discrete domains of the SAM or developing leaf primordia, we are using a technique called laser capture microdissection (LCM) that allows the isolation of specific cells within a tissue or organ, in combination with microarray analyses to compare global gene expression patterns between different cell populations. Expression profiles between the following cell types have been compared: (1) indeterminate stem cells in the SAM and determinate cells of newly initiated leaf primordia; (2) the epidermal (L1) and subepidermal (L2) layers of the SAM; and (3) SAMs and/or leaf primordia from developmental mutants and wild-type plants.

We have used this approach to compare the gene expression profiles in the SAM and first leaf primordia from wild-type and *leafbladeless1* (*lbl1*) mutants, which are defective in the biogenesis of tasiRNAs and consequently develop abaxialized leaves. We identified 352 genes whose expression levels are significantly altered in *lbl1*. Genes that are differentially expressed between wild type and *lbl1* include (1) components of the tasiRNA pathway, which suggests that potential feedback regulation in the tasiRNA exists; (2) known determinants of adaxial-abaxial polarity, which is consistent with the abaxialization of *lbl1* leaf primordia; (3) known developmental regulators; and (4) novel maize genes.

Classical surgical experiments along with our small RNA expression studies revealed a critical contribution for the L1 layer in adaxial-abaxial patterning. Interestingly, 31 genes that are differentially expressed between wild type and *lbl1* are also differentially expressed between the L1 and L2 meristem layers. These include genes predicted to function in auxin or lipid signaling. A role for such signaling pathways in organ polarity has often been postulated, but insights into these developmental mechanisms are lacking. *lbl1* acts in the incipient leaf to specify adaxial identity and to propagate leaf founder-cell recruitment. We identified 17 genes that are differentially expressed between wild-type and *lbl1* SAMs and between the incipient leaf and the SAM proper. Interestingly, we have identified three genes whose expression was altered in all three comparisons, making them very important candidates for further functional analysis.

We are currently in the process of validating key differentially expressed genes by real-time quantitative RT-PCR, followed by in situ mRNA localization, and the function of genes of particular interest will be determined using functional genomics resources available in maize and *Arabidopsis*. We are also taking a bioinformatics approach to identify potential targets of tasiRNAs among those genes that are up-regulated in *lbl1* apices. This project will provide a novel insight into gene networks controlled by tasiRNAs and involved in leaf polarity.

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BIOINFORMATICS AND GENOMICS

The reference version of the human genome, along with those of other animal and plant genomes, provides an unprecedented starting point for understanding the correlation between the structure and function of genetic material. Data-intensive projects in genomics, proteomics, and related fields have created an information explosion. Technologies such as DNA microarrays and high-throughput genotyping are transforming experimentation, creating an information bottleneck that multidisciplinary teams at CSHL are devising the technical and intellectual means to manage.

W. Richard McCombie and colleagues are using a new generation of sequencing instruments to determine variation in the genomes, transcriptomes, and epigenomes of animals and plants. As director of CSHL's Lita Annenberg Hazen Genome Sequencing Center, McCombie has led the effort to perform high-throughput sequencing of several organisms, including the plant *Arabidopsis*, the fission yeast *Schizosaccharomyces pombe*, and *Homo sapiens*. He and his team have successfully integrated next-generation sequencers capable of generating 6 to 8 billion bases of sequence data weekly. The new equipment is being devoted to the task of finding important points of sequence variation among individuals. Very high capacity translates into the ability to look quickly at many genes and their mutations in large numbers of people, work that is the predicate of associating mutations with the occurrence of illnesses such as schizophrenia (a current focus of McCombie's team) and cancer.

Lincoln Stein's lab is developing databases, data analysis tools, and user interfaces to organize, manage, and visualize the vast body of information being generated by genome scientists. One vitally important effort is the "Hap-Map" project, a database correlating single-nucleotide variations in the human genome sequence with their occurrence in three distinct global populations. This year, Stein's group, which serves as the Data Coordination Center for the project, incorporated into the database information about 40 susceptibility regions in 20 common human diseases, including diabetes, rheumatoid arthritis, Crohn's disease, coronary artery disease, and bipolar disorder. Another of the lab's projects is WormBase, a database of the genome and biology of the roundworm, *Caenorhabditis elegans*. The lab also manages and curates the Reactome and Gramene databases. Reactome, a compendium of fundamental biological pathways in humans, integrates peer-reviewed literature with genomic information, including a growing database of protein–protein interactions (now covering more than 2200 proteins). Gramene is an online comparative mapping resource for rice and other monocots that now incorporates the entire maize and *Arabidopsis* genomes, and partial popular and sorghum genomes. Stein's group has also created a Web site enabling members of the public to compare their own sequenced genomes to the human reference genome.

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 regulation, work that has manifold implications for the study of cancer and many other diseases. Their tools, used by investigators throughout CSHL and beyond, are designed to identify functional genetic elements within molecular sequences, as well as pathways that control and regulate gene expression. Recently, Zhang's group developed a series of computational tools that make use of statistical pattern-recognition techniques to identify exons, promoters, and posttranslational regulation signals in large genomic DNA sequences. They are also studying alternative splicing of exons and collaborate with other labs to characterize splicing enhancers and silencers. The lab's interest in epigenetics has resulted in development of an algorithm called the Human DNA Methylation Finder that predicts an important class of epigenetic changes in human DNA. This is one of several efforts in the lab to correlate epigenetic markers with transcriptional activity.

Using multidisciplinary approaches combining computational analysis, modeling, and prediction with experimental verification, Doreen Ware's lab seeks a deeper understanding of genome organization in plants. By looking comparatively across the genomes of plants in the same lineage—she focuses on cereals such as rice and sorghum—Ware and colleagues seek answers to such questions as: How are

genes conserved and lost over time? What are the fates of duplicated genes? Her team also studies gene regulation in plants, specifically looking at regulatory elements and microRNA genes and their targets. In addition to understanding basic biological questions, the group also helps create bioinformatic tools including public Web sites, open-source software, and analysis methods of value to the larger scientific community.

DEVELOPMENT OF NEXT-GENERATION SEQUENCING TECHNOLOGY

W.R. McCombie V. Balija S. Muller M. Regulski
L. Cardone L. Nascimento L. Spiegel
M. Kramer D. Rebolini Z. Xuan

This work was performed in collaboration with Greg Hannon at CSHL.

In January of 2007, the first of a new type of DNA sequencer was installed. These instruments—the Solexa 1G—are considered disruptive technology because their capabilities are so radically advanced relative to previous instruments. Although these instruments have incredible potential, it was clear that to use them effectively, they required substantial technical and logistical development. In addition, they would require the establishment of new procedures for sample preparation and data analysis.

In brief, the instrument works in the following manner. Sheared DNA is made blunt-ended and two different adapters are ligated to it. The DNA is size-selected (in the 150–300-bp range, typically) and slightly amplified. The DNA is then added to a flow cell, which is a microscope slide sealed on the top with eight small channels or lanes through it. The surface of the flow cell is densely coated with a random distribution of two oligos that are complementary to the two oligos added to the ends of the fragments. The fragments bind to the oligos on the surface and are amplified in situ. Following amplification, sequencing is carried out.

A primer corresponding to one of the adapters is added along with polymerase and the four nucleotides, each of which is modified with a blocking group to prevent further extension of the chain and a fluorescent dye corresponding to which base it is (A, C, G, or T). The reaction incorporates one base on each of the clusters of molecules on the surface depending on which base is next on the complementary strand of that molecule. The surface of the flow cell is then imaged during laser exposure and the image is stored. The blocking group and dye are then cleaved, and the process is repeated with another base addition. As sequencing progresses, the images after each successive base addition are compared and the base incorporated at each addition on a given molecule is determined. This gives the sequence of each fragment on the flow cell. The process typically yields readlengths of 32–36 bases. This is very short, but the power of the instrument comes in the number of reads it generates. A single run of about 3 days generates about 40 million reads. Thus, the total sequence produced is in excess of a billion bases per run.

The principal investigator's (PI) lab was one of the first to receive an Illumina instrument and also among the first to receive multiple Illumina instruments. Because our first instrument was installed in January 2007, we have been working to improve the results from this instrument. This has been a difficult task and presented a wide variety of challenges. At this time, while still striving to improve, we feel that we have built a significant experience and knowledge base that has led to a significant increase in our ability to operate these instruments at high efficiency.

In addition to in-house training, the PI and a bioinformatics specialist have received 1 week of training at Solexa, near Cambridge, U.K. The PI and collaborators as well as several people in the PIs lab participated in a course on next-generation sequencing (http://meetings.cshl.edu/courses/c-seqtech08.shtml). We have made significant progress in effectively using these new instruments. Figure 1 shows output by the group since the beginning of August.

BAR-CODED POOL SEQUENCED ON THE ILLUMINA

Sequencing throughput can be significantly increased by sequencing multiple samples in the same lane of a flow cell. The only prerequisite is the ability to distinguish among reads from different samples at the stage of data analysis. To this end, we synthesized a set of library adapters carrying identifying sequences, or bar codes. Each sequence consists of a unique combination of four nucleotides. This bar code is located on the 3′ end of one of the standard Illumina adapters (P5), between the region complementary to the sequencing primer and the 3′ terminal T.

The PAK4 region was amplified from six cell lines (5-kb amplicons across the ~55-kb gene region). The resulting products for each cell line were then pooled and sheared. Cell lines 1–5 were bar-coded with custom 4-bp bar-code adapters and cell line 6 was ligated with the standard Illumina adapter. All six cell lines were then

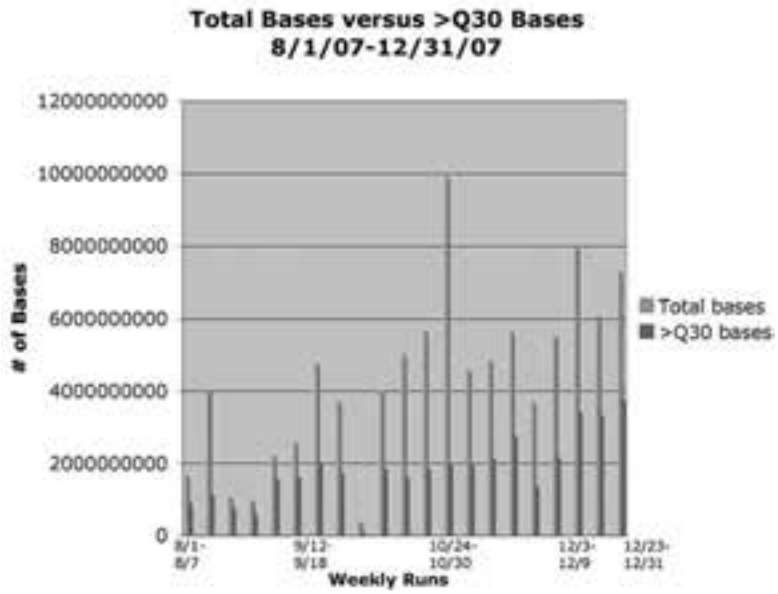

FIGURE 1 Comparison of total bases generated versus total high-quality bases for weekly Illumina runs from October 2007 through mid-January 2008. Note output drops during partial weeks around Thanksgiving and the Christmas and New Year's Day holidays.

pooled, loaded into six lanes of a flow cell, and sequenced for 36 cycles. Following base calling, the reads were sorted according to bar codes 1–5 or no bar code (standard adapter). The sequences with bar codes were trimmed to remove the first 5 bp (bar code plus T), and all the reads for each of the five bar-coded cell lines were then aligned to the PAK4 reference sequence using Eland. The results are shown in the table below. The results will allow us to take better advantage of the throughput of the instruments by allowing sample pooling within a lane.

Bar code	Total reads	Number of reads aligned with 0–2 mismatches	Percent of reads aligned with 0–2 mismatches
PAK4 BC 1	1433892	537235	37
PAK4 BC 2	1049330	494947	47
PAK4 BC 3	1102938	464935	42
PAK4 BC 4	1226655	446634	36
PAK4 BC 5	922861	530735	57

DATA ANALYSIS

Following sequencing, the data are analyzed through a data pipeline, which is a combination of the standard Illumina pipeline and customized software we have developed. The first part of the process is the standard image extraction and base calling of the Illumina pipeline. We then run this output into a customized program to compare the sequences to the reference sequence and call single-nucleotide polymorphisms (SNPs). This program, written by Zhenyu Xuan, takes into account both the sequence quality of the raw data (as assigned by the Illumina base caller) and the frequency of the variants present at a given residue. Both of these thresholds are adjustable and will likely be adjusted for different projects. Validation of a subset of alleles using orthologous methods such as ABI3730 sequencing is thus essential for the determination of proper thresholds for a given project. These steps and the results from their use are described below.

SNP DETECTION BY RESEQUENCED SHORT READS

We have developed an algorithm to identify SNPs based on Illumina-1G-sequenced short reads. We first mapped all reads to the reference genome by ELAND. On the basis of that alignment, we identified the frequently and reliably sequenced alleles' genotypes that differed from the reference genome, which we call nonreference alleles (NRAs), as the potential SNP candidates. On the basis of resequencing of the 55-kb human PAK4 gene region without using whole-genome amplification (WGA), we obtained 2.9 million reads that were uniquely mapped in the PAK4 region (using 32

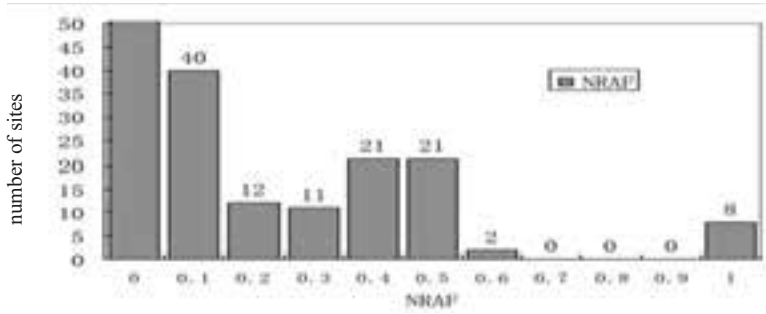

FIGURE 2 Nonreference allele frequency distribution as a function of PAK4 resequencing data. Only positions with at least 10x coverage of high-quality bases (quality score = 40) were calculated.

nucleotides in mapping). These reads covered 95.30% of the PAK4 region, which means that the average sequencing depth reached more than 1700x. Using quality scores calculated by the Illumina genome analyzer pipeline, we only chose the most reliably sequenced bases (with quality score of 40) to calculate the frequency of each NRA. We found that the distribution of NRA frequency (called NRAF) at each position of the genome followed a "W" shape (see Fig. 2), which had peaks at 0, 0.5, and 1.0. Peak 0 corresponds to non-SNP sites, whereas peak 1.0 corresponds to homozygous SNPs and peak 0.5 corresponds to heterozygous SNPs.

The DNA sample we used is a HapMap sample, NA11839, which has 21 known SNPs in the HapMap project. Table 1 lists the NRAF frequencies of all 21 SNP sites. All known SNP sites are covered more than 100 times by high-quality reads. We found that both known homozygous SNPs (rs1529712 and rs570278)

TABLE 1 Genotype and NRAF of known HapMap SNP sites in the PAK4 region

		NA11839		Non-WGA				WGA			
dbSNP_id	**Location**	**Genotype**	**Ref.**	**Genotype**	**Sum_all**	**Sum_NRA**	**NRAF**	**Genotype**	**Sum_all**	**Sum_NRA**	**NRAF**
rs1529712	44358807	TT	C	CT	453	452	0.9978	CT	133	132	0.9925
rs570278	44324653	GG	C	CG	2213	2207	0.9973	CG	1586	1581	0.9968
rs2085388	44320347	CT	C	CT	842	533	0.6330	CT	613	378	0.6166
rs692191	44314686	CT	C	CT	948	507	0.5348	CT	1233	587	0.4761
rs1549927	44319890	CT	T	TC	534	274	0.5131	TC	170	79	0.4647
rs17722300	44322375	AG	G	GA	1958	993	0.5072	GA	1389	655	0.4716
rs7257109	44354747	AG	G	GA	314	153	0.4873	GA	106	58	0.5472
rs12976130	44317640	CT	C	CT	1724	816	0.4733	CT	1950	902	0.4626
rs17795295	44323525	CT	C	CT	2100	993	0.4729	CT	2914	1358	0.4660
rs12611376	44318951	CT	C	CT	1304	603	0.4624	CT	510	245	0.4804
rs4803208	44316630	CG	C	CG	2496	1127	0.4515	CG	3606	1631	0.4523
rs513053	44332986	AG	A	AG	1131	495	0.4377	AG	633	282	0.4455
rs8107629	44324427	AG	G	GA	2409	1029	0.4271	GA	2504	1033	0.4125
rs12609418	44309292	CT	C	CT	717	262	0.3654	CT	547	142	0.2596
rs692475	44337574	CT	T	TC	586	207	0.3532	TC	118	46	0.3898
rs692257	44337190	CT	T	TC	721	248	0.3440	TC	187	62	0.3316
rs5828029	44319641	AT	A	AT	941	299	0.3177	AT	1299	390	0.3002
rs12611365	44319011	AG	A	AG	630	181	0.2873	AG	193	40	0.2073
rs692337	44329948	CG	G	GC	596	159	0.2668	GC	650	203	0.3123
rs529336	44345757	CT	C	C–	633	0	0.0000	CT	210	1	0.0048
rs691577	44348241	AG	A	A–	622	0	0.0000	AG	313	1	0.0032

HapMap-detected SNPs (21) are listed with their dbSNP id, genomic DNA location, and their reported genotype in sample NS11839. The genotypes determined by Illumina sequencing reads are listed together with the total reads coverage (sum_all), occurrence of NRA (sum_NRA), and NRAF (= sum_NRA/sum_all). Both results from sample prepared with or without whole-genome amplification are listed.

have NRAFs very close to 1.0, and 17 of 19 known heterozygous SNPs have NRAFs from 0.2 to 0.7. The other two heterozygous SNPs have NRAFs close to 0, although both have been sequenced more than 600 times. This may point to possible false-positive SNPs in the HapMap project, and we are doing capillary sequencing to verify our results.

On the basis of the knowledge from known SNPs, we used NRAF ≥ 0.2 for SNP calling and found 51 novel SNPs, including six homo-SNPs (NRAF > 0.9) and 45 hetero-SNPs (0.2 < NRAF < 0.9). We are also doing capillary sequencing to confirm them.

EFFECT OF WGA ON SNP DETECTION

We have also analyzed PAK4 resequenced data obtained from whole-genome amplified DNA (Qiagen REPLI-g Midi Kit). On the basis of our data, we did not find significant differences between samples prepared with or without WGA (see Table 1). In Figure 3, the NRAF detected at each site by the two preparation strategies are compared, and the correlation coefficient is 0.9758 for all sites covered at least 100 times and with NRAF > 0.05.

The detection of sequence variants in Illumina data presents a significant challenge given the error rates of current base-calling algorithms. However, we have a functioning pipeline in place, as well as the infrastructure for high-throughput resequencing with these next generation sequencers.

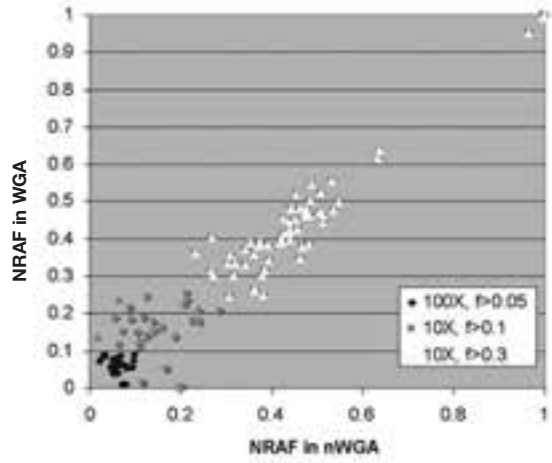

FIGURE 3 NRAF correlation for samples prepared with and without whole-genome amplification.

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M. Regulski and D. Rebolini

GENOME-SCALE DATABASES OF PATHWAYS, GENETIC VARIATION, AND EVOLUTION

L. Stein S. Avraham, S. Cain, N. de la Cruz, B. Faga, X. Feng, G. Gopinathrao, T. Harris, A. Kanapin, S. McKay, W. Spooner, P. van Buren, G. Wu, Z. Zha, W. Zhao

THE HUMAN HAPLOTYPE MAP

The International Human HapMap Project (www.hapmap.org) is an international project to map out regions of common genetic variability in the human genome by genotyping three major world populations across a large number of naturally variable sites. The resulting "haplotype map" will greatly reduce the cost of genetic association studies to find cancer susceptibility genes and other disorders with genetic components.

Our lab is a central participant in this project in our role as the Data Coordinating Center (DCC). We manage the central database for the project; allocate single-nucleotide polymorphisms (SNPs) to the 11 genotyping centers; coordinate data submission, quality checks, and quality control; and manage the public release of project data. The HapMap Web site, which was developed in our lab, describes the project in the four languages of the project participants (English, French, Chinese, Japanese, Yoruba) and provides access to the data both for bulk download and for interactive querying and browsing.

During 2007, we enhanced the HapMap Web site to provide tools to aid in designing and interpreting whole-genome association studies. In particular, we now provide a facility for merging the results from multiple independent association studies to greatly increase their aggregate power. We have also incorporated into the Web site information on a large number of common copy-number variations. In the past year, the HapMap Web site was used for several landmark whole-genome association studies that have identified novel genes implicated in type-1 and -2 diabetes, hypertension, rheumatoid arthritis, tuberculosis resistance, cardiovascular disease, inflammatory bowel disease, and other medically significant traits.

We are now adding support for the storage and analysis of individual genomes to the HapMap Web site, in anticipation of a new crop of "personalized genomes" that will appear in the next few years.

GRAMENE: A COMPARATIVE MAPPING RESOURCE FOR GRAINS

The Gramene database (www.gramene.org) is a comparative mapping resource for rice and other grains. Gramene allows researchers to compare the genetic and physical maps of the major monocot crops, namely, maize, barley, oats, sorghum, and wheat, to the emerging rice genomic sequence. This allows researchers to identify candidate genes in the rice genome that correspond to genetically mapped mutants and quantitative traits in the nonrice crop they are studying. Hence, the resource allows researchers studying traits in maize, barley, and so forth the benefit of genomic sequencing without waiting for the sequencing of these much larger genomes.

In addition to comparative maps, Gramene offers up-to-date genomic annotation of the rice genome, including both predicted and confirmed genes, and the current physical maps of rice and sorghum. We have mapped more than 1 million monocot expressed sequence tags (ESTs) to the rice genome, allowing gene predictions to be further refined based on cross-species comparisons.

A major milestone this year was the integration of genome mapping information on 11 wild rice species to the Gramene database. These species are adapted to a wide range of conditions, including semidesert and high-altitude sites. Our comparative maps provide a detailed view of how these genomes evolved and diversified from domestic life over a period of approximately 10 million years. By elucidating which regions were selected for during the divergence and adaptation of these species, we hope to provide information that will allow plant breeders to create new robust varieties of domestic rice.

We also used the information in the Gramene database to identify previously unknown microRNAs (miRNAs) in the rice, maize, and sorghum genomes. miRNAs are thought to be responsible for regulating key events during development and maturation. Our work provides the first window into how these genes evolve and diversify in plants.

WORMBASE: A RESOURCE FOR *C. ELEGANS* GENOME AND BIOLOGY

Our lab continues to be a major developer and maintainer of the WormBase database (www.wormbase.org),

an online information resource for the small free-living nematode, *Caenorhabditis elegans*. This organism is favored as a simple model animal because of its small genome size, experimental malleability, and well-understood cellular anatomy. WormBase is a curated model organism database developed as part of an international collaboration that includes the California Institute of Technology, Washington University at St. Louis, and the Sanger Centre. Our lab is responsible for the Web site, user interface, and software architecture for the project.

The resource, which is available to the public at www.wormbase.org, contains the complete *C. elegans* genome and key annotations, including predicted genes, alternative splicing patterns, oligonucleotide probes, and evolutionarily conserved segments. It also contains many other types of biological information, including the *C. elegans* cell pedigree, the organism's neuroanatomy, its genetic map, and the physical map from which the genomic sequence was derived.

During 2007, we used WormBase to sponsor a global gene prediction algorithm competition called NGASP (Nematode Gene-prediction ASsessment Project). This competition involved 16 computational biology groups to identify the combination of algorithms best able to predict novel genes from nematode sequences.

REACTOME

Reactome (www.reactome.org) is a collaboration with the European Bioinformatics Institute (EBI) and the Gene Ontology Consortium to develop a Web-accessible resource for curated information about biological processes.

Reactome is organized like a review journal. Bench biologists are invited to create modules that summarize a particular aspect of their field. Currently, summations include DNA replication, transcription, translation, intermediary metabolism, the cell cycle, RNA splicing, and hemostasis. Many more modules are under way. Modules are similar to minireviews, except that each paragraph of text is reduced to a series of logical assertions that is entered into a database of processes and macromolecules. The database is then used to drive a Web site that can be browsed like a textbook or searched with queries to discover pathways and connections.

During 2007, we brought the number of genes curated in Reactome to 2500 proteins, covering about 15% of the annotated portion of the human genome. We supplemented this data set with functional interaction data from microarrays, protein interaction experiments, and other sources to create an integrated network of functional interactions covering approximately 50% of the genome. This integrated network is the largest one published to date, containing

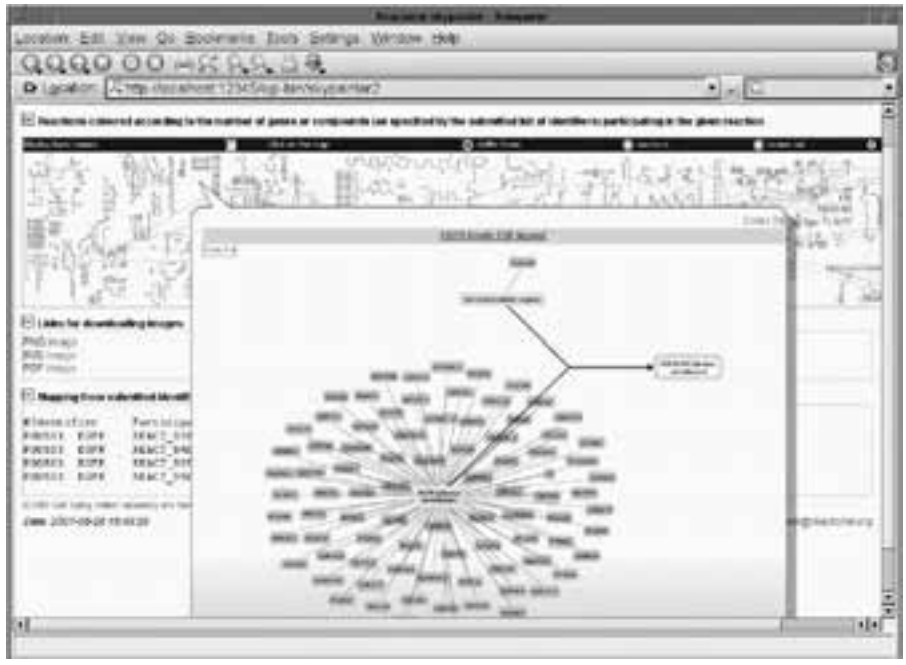

FIGURE 1 Reactome provides a tool for interpreting microarray expression studies. When a researcher uploads a list of genes that are coordinately up- or down-regulated, the tool will identify and display pathways that are overrepresented among those genes. Mousing over the affected pathways shows detailed information on which genes were over- or underexpressed and how they relate to each other.

more than 200,000 known and predicted gene interactions (Fig. 1). We are now validating the network and are developing methods to use it to interpret whole-genome association and copy-number variation experiments, thereby increasing the power of such studies.

GENERIC MODEL ORGANISM DATABASE PROJECT

In collaboration with the model organism system databases FlyBase, SGD, and MGD, the Generic Model Organism Database (GMOD) project is developing a set of database schemas, applications, and interfaces suitable for creating a model organism system database. These tools significantly reduce the time and expense required to create new databases to curate genomic information coming out of various model organism system sequencing projects. 2007 saw the establishment of GMOD-based model databases for chicken, cow, and *Escherichia coli*.

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Chengzhi Liang

PLANT GENOME ORGANIZATION, EVOLUTION, AND FUNCTION

D. Ware P. Canaran, B. Hurwitz, C. Maher, L. McMahan, A. Narenchania, S. Pasternak, L. Ren, W. Spooner, J. Stein, J. Thomason, P. Van Buren, K. Youens-Clark, L. Zhang, W. Zhao

COMPARATIVE GENOMICS: A MODEL FOR UNDERSTANDING FUNCTION THROUGH EVOLUTION AND GENOME ORGANIZATION

The cereal genomes are believed to have diverged from one another within the past 50 million to 80 million years (Ma). During this time, numerous genomic rearrangements have occurred in cereal genomes, such as segmental and tandem duplications, polyploidization, and transposition. This is especially true for maize, which has undergone tetraploidization and is known to have many recent modifications to its genome structure due to transposable elements. Previous studies have shown that regions of conserved protein-coding gene order between cereals represent functionally important regions and potentially conserved phenotypes.

The comparative mapping and integration of genomic sequence, genetic and physical maps, and phenotypes adds significant value to existing genome sequencing and mapping studies, by allowing the transfer of information across and within species. As part of this work, our group contributes to the infrastructure of Gramene (www.gramene.org). Gramene is a collaborative that leverages sequence and functional information from models and sequenced plant genomes for translational genomics in agriculture.

In the last year, our group, in collaboration with Dr. Lincoln Stein's group here at CSHL, completed several milestones on the Gramene project, including data updates, new acquisitions, analysis, and improvements in visualization and performance. Most notable was the addition of the Ensembl Compara pipeline to analysis tools, which included the development of protein-based gene trees and whole-genome nucleotide alignments. The gene tree allows researchers to rapidly identify conserved and nonconserved portions of plant genomes. Protein-based gene trees allow us to rapidly identify gene families that have remained the same, expanded, or contracted in different genomes. Because orthologous genes will in many cases have conserved function, researchers can leverage the knowledge gained from functional studies in one organism to infer or hypothesize function in organisms for which no functional information exists. These gene trees also allow researchers to look for structural changes to these genes. Structural polymorphisms can include single-amino-acid changes, insertions, or deletions, or even gain or loss of an entire exons. Observed differences may reflect artifacts of the annotation process or diversification between species, which could highlight potential new functions of these genes. The whole-genome alignments complement the protein-based comparisons identifying conserved noncoding sequence.

In addition to understanding the evolution and role of protein-coding genes, we are interested in understanding the evolution of noncoding genes and regulatory elements. Work in our group has focused on analyzing a class of noncoding genes called microRNAs (miRNAs). Specifically, we are interested in understanding if these noncoding genes have conserved gene order or synteny between species, as has been found among protein-coding genes. If the miRNA genes have remained intact in conserved genome locations, this would suggest that they also have maintained a similar functional role during the evolution of cereals. For this purpose, we have taken a two-prong approach, making use of both experimental and computational resources to characterize their genome location, gene structure, and expression patterns. In the last year, we physically mapped more than 30 miRNA genes in maize in collaboration with Georgia Davis at University of Missouri. Subsequent syntenic analysis with rice revealed that many of the miRNA genes are in regions of conserved gene order. The fact that these miRNA genes have remained intact, and in conserved genome locations, throughout the divergence of rice and maize suggests conservation of function. We took advantage of "next-generation" sequencing approaches to characterize miRNA (and small RNA?) transcripts in developing seedling and inflorescence tissues of maize and sorghum. These data sets were used to validate known miRNAs, discover paralogous genes, and analyze differential expression across species. Using 454 sequencing, we validated the expression of more than 55% of

previously identified maize miRNAs and 74% of known sorghum miRNAs; we also identified 31 maize and 14 sorghum paralogs, and five *trans*-acting small interfering RNAs. Approximately two thirds of these families were predicted to have conserved protein-coding targets across species. Differential expression analysis revealed that most miRNA sequences have conserved expression patterns at two key developmental stages, suggesting conserved regulatory roles.

Previous work characterizing the miRNAs has focused on the mature processed form of transcripts, but these small 20–22-nucleotide RNAs originate from much larger precursor transcripts. This past year, we have experimentally characterized these longer precursors using 5′–3′ race methods in maize and sorghum. Preliminary results found that many of these precursor structures are much larger than that predicted by the stem-loop structure which is typically used to annotate these noncoding genes. In addition, we found several examples in which one precursor structure appears to give rise to two mature miRNAs. We also found that several of the plant miRNA genes contain introns similar to those of protein-coding genes. By characterizing the primary transcript, we also established the start of transcription, opening the way forward to identifying proximal promoters and motifs responsible for regulating expression. By increasing our understanding of these small RNA regulatory networks, we will increase the potential of researchers and breeders to modify plant developmental profiles and responses to stress in order to positively meet the challenges of global environmental change and land allocation for agriculture in the upcoming years.

SEQUENCE AND ANALYSIS OF THE MAIZE B73 GENOME

From its domestication 8000 years ago in Central America to its position today as the world's leading harvested grain, *Zea mays* has had an important role in human civilization, providing food, animal feed, and biofuel. Maize also enjoys a long and distinguished history as a model organism owing to its rich diversity and tractable genetics. The complete sequence of the maize genome would therefore propel advances in basic research as well as agriculture and other industries.

The Maize Genome Sequencing Consortium was launched with a 3-year grant from the National Science Foundation to produce a complete sequence of the maize (B73) genome. At 2.5 Gb, the maize genome rivals mammalians in terms of size and is six times larger than rice, owing to its high content of retrotransposable elements. To meet the challenge of producing a draft sequence, the consortium took a hierachial approach, selecting a minimal tiling path of bacterial artificial chromosome (BAC) clones from a 20x fingerprint map and sequencing the BACs using a combination of shotgun and finishing of the unique regions of the BACs.

In addition to our lab, those of Drs. McCombie, Stein, and Martienssen here at CSHL are central participants in the Maize Sequence Consortium. Now in its third year, the project has produced complete sequences of 15,200 BAC clones comprising approximately 2 billion nonredundant bases, all available via GenBank. Our lab's specific contributions include aiding the BAC selections process, developing resources to assign unique regions of the genome for finishing, and providing automated annotations of the draft sequence. In the past year, we have enhanced visualizations and performance at the project Web site, produced annotations for the repetitive and genic regions of the draft sequence, and provided preliminary whole-genome comparisons with rice. The analysis reveals that the maize genome has been largely shaped by its history of tetraploidization, subsequent rearrangement, and duplicate gene loss. Gene annotations and comparative maps generated by this project are available at the project Web site Genome Browser (www.maizesequence.org) (Fig. 1).

ASSAYING MOLECULAR AND FUNCTIONAL DIVERSITY IN MAIZE

Dissection of complex traits in higher eukaryotes still remains one of the major challenges in biology. Currently, the dissection of complex traits involves either the identification of large genomic regions or the testing of genes one at a time. At the heart of both approaches is a requirement for the construction of appropriate populations within which segregating markers can be easily scored. To address this issue in maize, we are participating with two projects: Maize Diversity and High Scorable Markers for Trait dissections.

The Maize Diversity project's main goals are to understand molecular diversity of maize and teosinte, develop resources for functional trait dissection, map quantitative trait loci (QTL) across maize and teosinte, and evaluate functional variation at candidate genes. In 2007, we updated the available data sets that we maintain as part of the public portal for the project data Panzea (www.panzea.org) and improved visualization tools. One such example of this was building a framework for the display of diversity data using Google™ Maps. This allows researches to view the genotype data

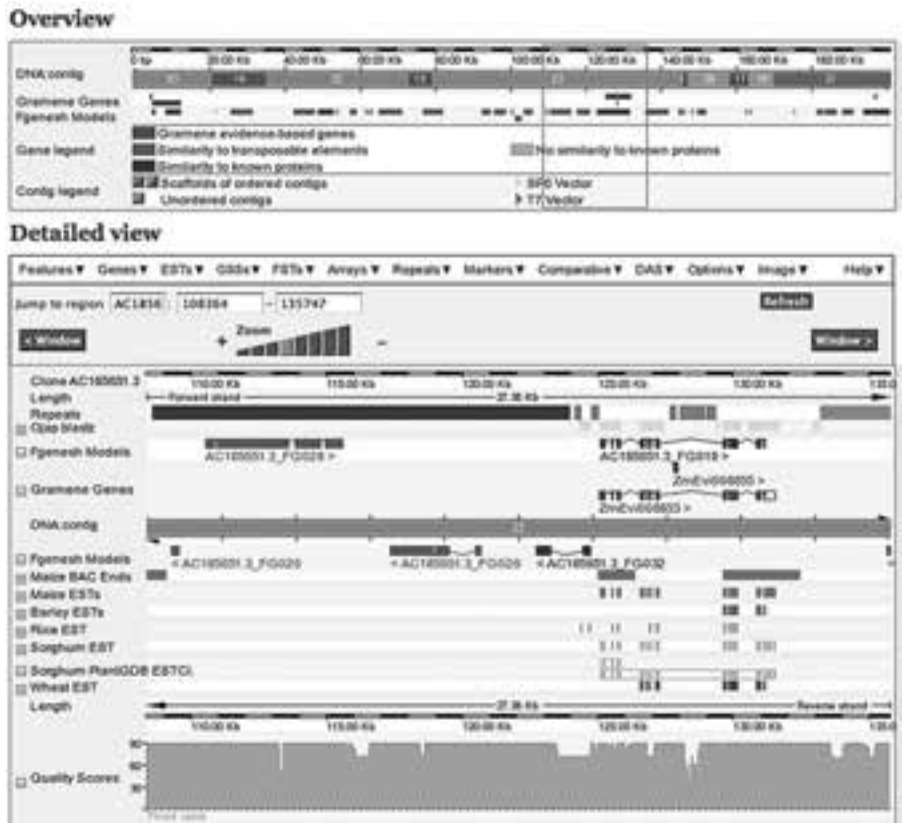

FIGURE 1 Sample of a view of an annotated maize BAC available from www.maizesequence.org.

in context to geographical distribution of the germplasm. By integrating the geographical and genotype information, researchers can clearly see patterns of loss or gain of diversity in candidate genes. In addition to the development of computational tools for the project, our group is interested in understanding the role miRNA genes have had in the process of domestication and improvement. To address this question, our group identified candidate miRNA genes that have been incorporated into the genotyping platform provided by the project. Preliminary results in 2007 suggested that a few of these miRNA genes show a strong correlation with several traits scored in these populations.

The second project builds upon resources developed in the Maize Diversity and Maize sequencing project to identify 1–2 million SNPs in maize (*Zea mays* ssp. mays) by generating genome reduction libraries in the diverse maize inbred lines. Importantly, these lines are parents of the 5000 recombinant inbred lines (RILs) that form the basis of the world's largest ongoing complex trait dissection project. In combination with this unparalleled QTL mapping resource, these SNP alleles will enable the resolution of numerous QTL down to the gene level.

In the past year, our group and Dr. McCombie's have focused on developing robust analysis pipelines for handling resequencing data sets for SNP discovery. On the basis of preliminary analysis of the 454 and Solexa data sets, we have decided to focus on the Solexa sequencing platform, sacrificing longer read lengths for increased genome coverage. In the next year, we anticipate having 3× coverage per line, for more that 30× coverage across ten lines to generate polymorphisms at approximately 400,000 locations in the maize genome. This resource combined with the germplasm and genome sequence described above will provide the most robust resource for complex trait dissection in any higher eukaryotic organism.

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Lifang Zhang

Apurva Narechania

COMPUTATIONAL GENOMICS

M.Q. Zhang J. Rosenfeld X.W. Wang C. Zhang
J. Silverman H.Y. Weng X.Y. Zhao
A.D. Smith Z.Y. Xuan

In the last year, Dustin Schones finished his Ph.D. and went on to a postdoctoral fellowship at the National Institutes of Health/National Heart, Lung, and Blood Institute. Visiting professor Fei Li returned to China, and Dr. Xiaoyue Zhao joined the Biotech company Bionovo in the Bay area. We had Josh Silverman as an URP student from Duke University and H.Y. Weng as a summer intern from Taiwan. Visiting Ph.D. student Xiaowo Wang joined the lab in the fall from Tsinghua University in China. The year 2007 has been very productive, despite the severe budget cuts from the government.

Transcriptional Gene Regulation

NEW METHODS FOR IDENTIFYING AND ACCESSING STATISTICAL SIGNIFICANCE OF *CIS*-REGULATORY ELEMENT MOTIFS

In collaboration with Tsinghua University, a one-class support vector machine (SVM)-based method OSCAR has been developed (Jiang et al. 2007) that can outperform position weight matrix (PWM)-based methods. For better accessing *cis*-element motif significance, we developed a new method that allows users to compute the *p* values for a motif or a module quickly using empirical *p* values compiled from our CSHLmpd promoters (Schones et al. 2007). We have also derived mathematically rigorous *p* values for Markov motif models, proved the problem is NP-hard (nondeterministic polynomial-time hard), and implemented it in a fast algorithm (Jiang et al. 2007) in collaboration with the University of Waterloo and Tsinghua University.

NOVEL CORE-PROMOTER PREDICTION ALGORITHMS

Two new state-of-the-art human core-promoter prediction algorithms have been developed. One is *CoreBoost* (Zhao et al. 2007) based on Random Forest and Tree Boosting technology. The other is based on a newly proposed multi-objective-simulated annealing-based optimization method, Archive Multi-objective-simulated Annealing (*AMOSA*) (Wang et al. 2007) in collaboration with the Indian Statistical Institute and Tsinghua University. Both have further improved the accuracy of detecting TSSs (transcriptional start sites), including those in non-CpG promoters.

MAPPING OF TISSUE-SPECIFIC PROMOTERS AND *CIS*-REGULATORY ELEMENTS

Using chromatin immunoprecipitation (ChIP)-chip with high-density genomic tiling arrays, we have mapped active promoters in mouse embryonic stem cells and several other adult organs in collaboration with Nimblegen and the University of California at San Diego (Barrera et al. 2008). Equipped with our suite of motif discovery tools, we further extended our general study of tissue-specific transcription-factor-binding site (TFBS) motifs (Smith et al. 2007) to more experimental systems in collaboration with various wet-bench scientists. We have also investigated transcriptional regulation pathways in more detail in hair follicles (Mignone et al. 2007, a collaboration with the Enikolopov lab here at CSHL), in adipocytes (Eguchi et al. 2008, a collaboration with Beth Israel Deaconess Medical Center), in blood cells (Yang et al. 2007, a collaboration with the University of California, Los Angeles), and in lung (Martinez et al. 2007, a collaboration with Lovelace Respiratory Research Institute).

MOST CPG ISLANDS MAY BE PROTECTED FROM DNA METHYLATION BY ZINC FINGER PROTEINS

After developing algorithms for predicting CpG island methylation (Fang et al., *Bioinformatics 22:* 2204–2209 [2006]), in collaboration with Tsinghua University, we further studied DNA sequence characteristics in the boundary regions of methylation-resistant CpG islands and discovered that certain classes of zinc finger proteins (e.g., CTCF, Sp1, MAZR, ETF, AP2, SPZ1, KROX, and NF-κB) may have important roles in protecting such islands from methylation in human brains and other tissues (Fan et al. 2007). This is consistent with our recent genome-wide CTCF mapping data (Kim et al. 2007).

Posttranscriptional Gene Regulation and Miscellanea

IDENTIFICATION OF SYNAPTIC TARGETS OF *DROSOPHILA* PUMILIO

This is a multiyear project (Chen et al. 2008) mainly supported by Dart Neuroscience Inc. and has just been completed as a collaboration with the Dubnau, Krainer, and Tully labs here at CSHL. *Drosophila* Pumilio (Pum) protein is a translational regulator involved in embryonic patterning and germ-line development. Recent findings demonstrate that Pum also has an important role in the nervous system, both at the neuromuscular junction (NMJ) and in long-term memory formation. In neurons, Pum 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 characteristics, the biologically relevant targets of Pum in the nervous system remain largely unknown.

We hypothesized that Pum 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 to predict Pum targets among mRNAs whose products have synaptic localization. We then used both in vitro binding and two in vivo assays to functionally confirm the fidelity of this informatics screening method. We find that Pum 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. Finally, we show that the endogenous *dlg1* mRNA can be regulated by Pum in a neuronal context, the adult mushroom bodies (MB), which is an anatomical site of memory storage.

ANALYSIS OF RNA SPLICING REGULATION AND EVOLUTION

During the process of building a comprehensive splicing database (http://rulai.cshl.edu/dbCASE) from de novo mRNA/EST (expressed sequence tag) genome alignments, we have discovered a new class of pre-mRNA splice sites, dubbed "dual-specificity splice sites," which can function as either 5′ or 3′ splice sites in vivo (Zhang et al. 2007). A careful statistical study of alternative splicing (AS) in human and mouse indicates that many of the AS products (e.g., in *DBEST*) are likely to be nonfunctional; evolution favors frame-preserving events and smaller-size exons (Zhang et al. 2007).

STUDIES ON miRNAS, FEMALE GERM LINE, AND AUTISM

In collaboration with Tsinghua University, we have identified a comprehensive set of phylogenetically conserved miRNA *cis*-regulatory elements across 12 *Drosophila* species (Wang et al. 2008). This is one of the pioneer studies on genome-wide miRNA gene promoter architectures.

Working with the Hannon lab here at CSHL, Z.Y. Xuan studied miRNA regulation in a specific experimental system: the p53 tumor suppressor gene network in the mouse (He et al. 2007) and also investigated the critical role of Dicer in the female germ line during meiosis (Murchison et al. 2007). Working with Dr. Wigler and other investigators here at CSHL, X.Y. Zhao has proposed a unified genetic model for sporadic and inherited autism (Zhao et al. 2007).

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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 three 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.

Previous Cold Spring Harbor Laboratory Fellows Adrian Krainer (1986), Scott Lowe (1995), and Marja Timmermans (1998) are now members of the faculty at the Laboratory; Adrian is also the Director of the Fellows program. After nine years at the Laboratory, Carol Greider (1988) left to join Johns Hopkins University School of Medicine, where she is now the Daniel Nathans Professor and Director of Molecular Biology and Genetics. Eric Richards (1989) is now a Professor in the Department of Biology at Washington University in St. Louis. After completing his fellowship, David Barford (1991) returned to the Laboratory of Molecular Biophysics at Oxford University and is now a Professor of Molecular Biology at the Institute of Cancer Research in London. Ueli Grossniklaus (1994) was a member of our faculty before leaving to become a Professor at the Institute of Plant Biology, Universität Zürich, Switzerland. Térence Strick (2000) left at the end of his fellowship to become a Group Leader at the Institut Jacques Monod, Centre National de la Recherche Scientifique and Universités de Paris VI and VII.

The Laboratory's three most recent CSHL Fellows are Gilbert (Lee) Henry, who joined the Laboratory in 2000, and Patrick Paddison and Ira Hall, both of whom joined the Laboratory in 2004. Their reports are listed below. Lee joined us from Doug Melton's laboratory at Harvard University, where he earned his Ph.D. for studies on *Xenopus* development. As a Fellow, Lee studied the molecular biology of taste receptor cell differentiation. Ira was a graduate student at the Watson School of Biological Sciences, where he worked in Shiv Grewal's lab on the relationship between RNA interference and chromosome dynamics. As a Fellow, he studied mammalian genomic instability and plasticity and characterized the contribution of DNA copy-number polymorphisms to genetic variation in inbred mouse strains. Patrick was also a graduate student at the Watson School of Biological Sciences, where he worked in Greg Hannon's lab on the development of RNA interference libraries. As a Fellow, Patrick has been using RNA interference technology to establish the genetic requirements for embryonic stem cell self-renewal and differentiation.

Both Lee and Ira moved to Virginia this year: Lee left in July to work on a project headed by Thomas Südhof at Howard Hughes Medical Institute's (HHMI) Janelia Farm in Ashburn; Ira, who left in November, is now Assistant Professor in the Department of Biochemistry and Molecular Genetics at the University of Virginia in Charlottesville.

I. Hall
L. Henry
P.J. Paddison

SWI/SNF-dependent Dismantling of Pluripotency Networks in Embryonic Stem Cells

P.J. Paddison, C. Cormier, T. James

Embryonic stem cells (ESCs) are cell lines derived from the inner cell mass (ICM) of blastocyst-stage mammalian embryos (Evans and Kaufman, *Nature 292:* 154 [1981]; Martin, *Proc. Natl. Acad. Sci. 78:* 7634 [1981]; Thomson et al., *Science 282:* 1145 [1998]). They can grow indefinitely in culture and give rise to cells of all three embryonic germ layers as well as germ cells (Keller, *Genes Dev. 19:* 1129 [2005]). For this reason, ESCs hold great promise for regenerative medicine, where human ESC derivatives might be used in cell replacement therapies.

Nanog is an atypical homeobox domain transcription factor that is one of several known key regulators that are required to maintain pluripotency of ESCs (Chambers et al., *Cell 113:* 643 [2003]; Mitsui et al., *Cell 113:* 631 [2003]). Current models posit that Nanog, along with factors such as Oct4 and Sox2, functions in cross-regulatory networks that collectively repress differentiation-inducing genes and activate those that are required for self-renewal. To find regulators of self-renewal and lineage specification, we used an RNA interference (RNAi) screening approach to find modifiers of Nanog expression in ESCs.

Confirming the validity of the screening approach, many previously known and recently identified players in ESC self-renewal scored as Nanog activators, including genes involved in major signaling pathways required for pluripotency (i.e., LIF-Jak/Stat, Wnt-Cttnb1, and Bmp-Smad4), as well as pluripotency transcription factors (e.g., Esrrb, Oct4, Sall4, Sox2, and Rif1). Among genes scoring as Nanog repressors, we found the only known direct, physiological repressor of Nanog, Tcf3, as well as negative regulators of Jak/Stat, Cttnb1, and Smad4 signaling and Raf1, which has known roles in mESC differentiation. Chief among the novel screen hits in this category were four core members of the Brg1-associated SWI/SNF chromatin remodeling complex.

SWI/SNF regulates gene expression by locally affecting chromatin structure to control access of transcription factors and basal transcription machinery. Therefore, SWI/SNF-dependent repression of ESC self-renewal genes could result from direct regulation or from indirect regulation, where SWI/SNF promotes expression of secondary factors, which, in turn, repress self-renewal gene expression. To distinguish between these possibilities, we compared the Smarcc1/Baf155-bound chromatin regions of the mouse genome in ESCs with Smarcc1-dependent changes in gene expression during ESC differentiation. Genes displaying both Smarcc1 binding and altered gene expression in its absence are candidates for direct regulation by the SWI/SNF complex. From this analysis, we find that the SWI/SNF complex has critical roles in ESC lineage specification as a corepressor of self-renewal genes, a coactivator of differentiation/lineage specific genes, and a coordinator of developmental patterning.

PUBLICATIONS

Paddison P.J. and Vogt P.K., eds. 2007. *RNA interference* (*Current topics in microbiology and immunology),* Springer-Verlag, Heidelberg, Germany.

In Press

Paddison P.J. 2008. RNA interference in mammalian cell systems. *Curr. Top. Microbiol. Immunol.* **320:** 1–19.

Investigation of DNA Copy-number Fluctuation using Genomic Microarrays

I. Hall, C. Egan

We are interested in pursuing a genome-wide examination of genetic instability and plasticity and in characterizing the contribution of DNA copy-number differences to natural variation. The arrangement and copy number of chromosomal segments may vary among species, strains, and individuals, and spontaneous DNA rearrangements are recognized to be causal in the clonal evolution of cancers and in the etiology of certain human diseases. Ancestral sequence relationships within and between mammalian genomes indicate a major architectural role for duplication and deletion in shaping genomes over evolutionary time, but less is known about how these processes contribute to genetic variation across more rapid time scales within normal populations and organisms. We aim to use the laboratory mouse as a model system to investigate the fundamental properties of DNA copy-number fluctuation in mammals utilizing a form of comparative genome hybridization termed representational oligonucleotide microarray analysis (ROMA) (Lucito et al., *Genome Res. 10:* 1726 [2000]).

Internal relationships within sequenced genomes show that a significant portion (2–5%) of chromosomal DNA in mammals is contained within segmental duplications (defined as stretches of DNA within a single genome >1 kb in length and >90% identical to one another), and duplicative events underlie the historical amplification and diversification of many important gene families. Sequence comparisons are essential to identify ancient copy-number changes, but they are not well suited to detect recent and ongoing genetic events because a complete genome sequence (1) is usually derived from a single individual, (2) relies upon assembly methods that are confounded by large identical repeats, and (3) is still impractical to attain for large genomes. Traditional molecular techniques are inevi-tably limited (and directed) to a small number of loci, and cytogenetic methods lack sufficient resolution to detect most changes.

ROMA allows for the simultaneous detection of DNA copy-number variants (CNVs) and restriction-fragment-length polymorphisms (RFLP) between any two related DNA samples through comparative hybridization of simplified genomic representations to high-density oligonucleotide microarrays. This technique assays a large portion of the genome in a relatively unbiased manner and requires only that a complete genome sequence exist

for the organism in question. A number of recent studies have catalogued extensive copy-number differences between humans, indicating that CNVs may account for a substantial fraction of existing genetic variation.

The apparent evolutionary importance and contemporary prevalence of segmental copy-number variation raise fundamental questions: How often do new CNVs arise? Do CNVs arise through a random process? Does copy number generally reflect the ancestry of a locus? What are the prevalent mechanisms of duplication and deletion? Are different chromosomal regions or classes of DNA sequences more variable than others? Do distinct cells or cell types of an organism contain the same genetic material?

The laboratory mouse is an ideal model system for such investigations in that the human and mouse genomes appear to have been shaped by similar mutational forces, and the mouse offers the significant technical advantages of controlled crosses, experimental manipulation, and a known breeding history.

GENETIC DIVERSITY IN INBRED MICE

During the past century, a large number of phenotypically diverse inbred strains have been derived from a small number of founder mice through brother-sister mating. Because these founders were mixed descendants of Asian and European subspecies, each modern inbred line contains a unique, recombinant mixture of chromosomal segments of distinct genetic origin. These genetic differences are thought to underlie the many interesting physical and behavioral traits for which modern strains vary.

To gain an understanding of the current genetic composition of the laboratory mouse and to evaluate the utility of ROMA as a tool for mouse genetics, we have profiled the genomes of a number of commonly used inbred strains. Using a microarray containing approximately 83,000 probes, we have identified about 15,000 ROMA polymorphisms from 12 strains. More than one third of these markers can be observed in a single strain comparison, and further investigation with independent methods indicates that about one fifth of them are CNVs (the remainder are RFLPs and SNPs). These markers are distributed throughout the genome in a punctate, highly nonrandom fashion, and in collaboration with S. Sridhar (Carnegie Mellon University), we have developed a segmentation algorithm to identify ancestrally divergent haplotypes and CNVs within our data (this model is based upon previous work by L. Muthuswamy and M. Wigler here at CSHL). We have compared ROMA polymorphisms to a curated set of 3.8 million single-nucleotide polymorphisms (SNPs) and found that although the majority of ROMA differences appear to be ancient in origin and mirror the distribution of SNPs, significant genomic changes have occurred since the establishment of inbred lines in the early part of the 20th century.

GENOME STABILITY AND PLASTICITY

To assess the importance of segmental duplication and deletion in generating de novo genetic variation, we are examining genome stability through normal cycles of somatic development and germ-line transmission in the laboratory mouse.

A description of the genetic variation normally present in somatic cells is important for a few major reasons. Each cancer is thought to begin with a single cell that has diverged from its relatives, and the accumulation of mutations is thought to be a cause of aging. DNA rearrangement is also an established regulatory mechanism; our own bodies generate a staggering diversity of immunological molecules precisely through such "mutagenic" processes, and more simple genetic systems (such as various yeasts and bacteria) utilize reversible DNA rearrangements to make heritable transcriptional decisions. It is not known how common such mechanisms are in mammals. In collaboration with CSHL's J. Hicks (the Wigler lab) and K. Eggan (Harvard University), we are pursuing a survey of somatic cell diversity in humans and mice. We hope that these studies result in an estimate of clonal variegation in mammals and help to identify unstable or hypervariable regions of the genome.

Mutation in the germ line is the basis for the introduction of new alleles to a species and is relevant to our understanding of evolution, population structure, and sporadic human disease. Previous estimates of the germ-line mutation rate have relied upon the observation of spontaneous loss of function at a small number of genes and do not adequately distinguish between classes of mutations. Rates of segmental duplication and deletion have been examined at just a few loci. Our analysis of genetic diversity described above utilized multiple individuals from each strain and thus represents a test of the purportedly homozygous and uniform nature of the inbred mouse genome. We found that inbred mice are indeed very inbred but are rarely identical. We did not observe residual heterozygosity of ancestral genetic variation, but we often encountered one or more spontaneous CNVs between closely related individuals of the same inbred strain. This indicated that CNVs might arise at a high rate and that it might be possible to study this process directly.

During the past year, we completed a study of spontaneous DNA copy-number change across the entire mouse genome. We collected pedigreed mice from many sepa-

rate colonies of the C57BL/6 (B6) inbred strain, each bred independently at different institutions around the world. Using information obtained from breeders, we constructed a genealogy spanning approximately 966 generations of inbreeding. We then used ROMA to identify 38 de novo CNVs among the B6 colonies.

To our surprise, almost half of the de novo CNVs arose multiple times within distinct lineages: Eight of 38 CNVs arose once within B6 colonies but were also variable among other classical inbred mouse strains, and ten CNVs arose multiple times within closely related B6 colonies. Thus, significant changes in DNA copy number occur over relatively short timescales, and new segmental variation is often the product of recurrent mutation. The recurrent CNVs that we identified range in size from 4 kb to 4 Mb, affect 43 known genes, and fluctuate in copy number at rates of about 1×10^{-2} to 1×10^{-3}/locus/generation—unprecedented rates for large structural mutations. We used high-resolution tiling arrays to map CNV breakpoints to approximately 500-bp resolution and found that they recur through a spatially precise mechanism. Moreover, breakpoints are often found within repetitive elements.

Given that we have significantly underestimated the prevalence of recurrent CNVs and that many of those we identified contain genes for which hypervariability might be beneficial to a population (i.e., factors involved in immunity, reproduction, and cognition), we believe that recurrent structural mutation will prove to be a widespread and phenotypically relevant process for creating genetic variation in mammals.

PUBLICATIONS

Egan C.M., Sridhar S., Wigler M., and Hall I.M. 2007. Recurrent DNA copy number variation in the laboratory mouse. *Nat. Genet.* **39:** 1384–1389.

Structural and Functional Studies of the Vertebrate Taste Bud

L. Henry, M. Siddiqui

We are interested in the molecular biology of taste receptor cell differentiation. The mammalian taste bud is a dynamic structure consisting of mitotically active progenitor cells at its periphery and differentiated taste receptor cells within its core. The basic goal of our research is to understand how the daughters of the progenitors select a particular fate. Because different receptor cells are known to respond to different classes of tastants, we hope that understanding this differentiation process will help to illuminate details of both taste bud structure and function. Toward this end, we have undertaken an exhaustive effort to define at the level of transcription the cellular diversity of the murine taste bud. To do this, single-cell profiling is being used to analyze the transcriptomes of taste receptor and progenitor cells.

I have completed the design of a novel means of amplifying mRNA from limiting amounts of starting material. The method involves the use of a solid-phase magnetic bead system wherein a 330-nm paramagnetic bead is coupled to an mRNA capture oligonucleotide. There are two novel aspects of the approach. First, the beads are approximately ten times smaller in diameter than beads used for similar purposes by other researchers. This decrease in diameter sounds modest; however, a tenfold decrease in diameter creates a tenfold increase in surface area and a 1000-fold increase in bead concentration per unit volume. Both of these features enhance the ability of the beads to bind to mRNA. In addition, because nanometer sized beads settle slowly, complicated agitation schemes are not required during enzymatic manipulations, including PCR (polymerase chain reaction) which can be done directly on the beads. The second novelty is the development of hybrid DNA/RNA capture oligomers that specifically prime cDNA synthesis while blocking extension in the presence of other needed polymerases.

This advance greatly reduces a background problem associated with cDNA amplification schemes where the ends of the cDNA are adapted with common sequences to allow PCR amplification. In addition, the approach is universal in that it is possible to generate a single-stranded RNA-based probe or a double-stranded DNA-based probe. We have begun screening amplified probes using Affymetrix microarrays as a readout and are at the initial stages of characterizing the transcriptomes of individual taste receptor cells.

AUTHOR INDEX

Aksoy C., 35
Alex G., 81
Alexander A., 154
Algieri C., 81
Alston N., 75
Andrews P., 139
Angiolillo J., 35
Aranda V., 88
Aravin A., 60
Archibald H., 92
Asari H., 149
Auletta T., 71
Avraham S., 178

Bagchi A., 75
Balija V., 174
Bambino K., 43
Bayer M., 154
Bell K., 145
Bender H.S., 60
Benitez Y., 161
Bergami K., 88
Bestman J., 108
Bialucha C., 67
Biot C., 92
Bish R., 45
Blum A., 111
Boehm J., 136
Boettcher S., 60
Boettner B., 101
Bohland J., 139
Boivin B., 94
Bokil H., 139
Bolduc F., 145
Bommert P., 161
Bouyer D., 161
Brennecke J., 60
Bric A., 67
Bronson K., 108
Burgess D., 67
Burgos K., 108
Byrnes J., 71

Cai C., 77
Cain S., 178
Campbell R., 147
Canaran P., 181
Canela A., 60
Cardone L., 174
Chakraborty A., 55
Chakraborty S., 81
Chang A.-Y., 157
Chang K., 60
Chatterjee S., 88
Chaudhary F., 94
Cheloufi S., 60
Chen B., 106
Chen K., 161
Chen M., 99
Chen S., 71
Chen Y., 145
Chen Y.-C.M., 47
Chiang H.-C., 151
Chicas A., 67
Chien Y., 67
Chitwood D., 167
Chiu S.-L., 108
Chklovskii D., 106
Cho H., 99
Claudio-Campos K., 60
Cline H., 108
Collins G., 55
Cook K., 81
Courtney L., 154
Cox H., 145
Cressy M., 111
Cutsail G., 38
Cutter N., 71
Czech B., 60

da Silva J.S., 108
Daulny A., 55
Davis E., 60
Dawkins-Pisani N., 136
de la Cruz N., 178
DeBlasio S., 161
Declerck M., 167
Demas J., 108
Demir E., 147
DePre K., 157
Dickins R., 67
Diggins K., 67
Dubnau J., 111
Duelli D., 86
Dus M., 60

Encinas J.M., 115
Enemark E., 35
Enikolopov G., 115
Erlich Y., 60
Esposito D., 81
Ewald R., 108

Faehnle C., 35
Faga B., 178
Fang Y., 47
Feierstein C., 131
Fejes-Toth K., 60
Felsen G., 131
Feng X., 178
Fenoglio S., 92
Fernandez-Marco C., 167
Ferreira P., 157
Finigan P., 53
Fregoso O., 38, 45
Fu Y., 121
Furukawa H., 119

Gallavotti A., 161
Galli M., 154
Gao D., 43
Geng F., 55
Genkin A., 106
Gierszewska M., 90
Gingeras T., 60
Girard A., 60
Gopinathrao G., 178
Gottesman A., 86
Graf R., 55
Grubor V., 81
Gruntman E., 157
Guo M., 167
Guo X., 75

Haase A., 60
Hagiwara H., 136
Hahn M., 43
Hakker I., 151
Hall I., 188
Han J.-J., 157
Hannon G., 60
Haque A., 94
Harris T., 178
Hastings M., 38
He H., 35
He L., 60
He M., 121
He X., 60
Hearn S., 47
Hemerley A., 53
Henry L., 188
Hicks J., 71, 81
Hippo Y., 67
Hiramoto M., 108
Ho I.S., 151
Hodges E., 60
Hoek M., 53
Hotta I., 60
Hrecka K., 90
Hromadka T., 149
Hsieh H., 136
Hu C., 167
Hu H., 136
Hua Y., 38, 145
Huang Z.J., 121
Hübner M., 47
Hufnagel L., 81
Hurwitz B., 181

Ibarra I., 60
Irvine D., 157

Jackson D., 161
Janas J., 101
Jankowska A., 60
Jaramillo S., 149
Jensen M., 38
John K., 101
Johnston R., 161
Joshua-Tor L., 35
Jurgensen C., 111

Kamalakaran S., 71
Kanapin A., 178
Kandasamy R., 81
Kara N., 53
Karakas E., 119
Karginov T., 60
Karni R., 38
Kawakami H., 53
Kendall J., 81
Kepecs A., 125, 131
Kessels H., 136
Keyes W., 75
Khalil D., 55
Khan S., 71
Kim S.-J., 94
Klein M., 136
Kloc A., 157
Kobayashi A., 101
Kopec C., 136
Kotkin S., 161
Koulakov A., 129
Krainer A.R., 38
Kramer M., 174
Krasnitz A., 81
Krizhanovsky V., 67
Kuhlman S., 121
Kumar P.R., 35
Kumaran R.I., 47
Kurland J., 55
Kusenda M., 143
Kvitsiani D., 121, 125
Kwofie M., 90

Lara M., 60
Lazarus M., 121
Lazebnik Y., 86
Lee B.-H., 161
Lee S.-Y., 108
Lee Y.-H., 81
Lee-Osbourne J., 108
Leonardo J., 60
Leotta A., 81
Leung A., 55
Levy D., 81
Li B., 136
Li H., 108
Li J., 47, 108
Li L., 94
Li W., 75, 111
Lima-Mainen S., 149
Lin G., 94
Lin J., 139
Lin P., 143
Lin W., 145
Linsted T., 92
Liu Q., 77
Liu X., 50
Liu Y., 38
Liu-Sullivan N., 77
Locke S., 157
Lodha M., 167
Lowe S., 67
Lu J., 121
Lucito R., 71
Lucs A., 88
Lukowitz W., 154
Lum E., 71

Ma B., 67
Maher C., 181
Mainen Z.F., 131
Makarov V., 143
Makino H., 136
Malhotra D., 143
Malinow R., 136
Malone C., 60
Mamiya A., 151
Manche L., 38
Marks S., 81
Marran K., 60
Martienssen R., 157
Mazurek A., 53
McCarthy S., 143
McCombie W.R., 174
McCurrach M., 67
McDonnell K., 43
McJunkin K., 67
McKay S., 178
McMahan L., 181
Melgar K., 92
Mellick A., 43
Meth J., 81
Michurina T., 115
Miething C., 67
Mills A., 75
Mishchenko Y., 106
Mitelheiser S., 53
Mitra P., 139
Mittal V., 43
Mizuno H., 60
Mohanty A., 161
Molaro A., 60
Mosquera M., 60
Mu D., 77
Muller S., 174
Murakami M., 131
Murchison E.P., 60
Muthuswamy L., 81
Muthuswamy S., 88
Myers M., 45

Nadif Kasri N., 101
Nakamura T., 47
Narenchania A., 181
Nascimento L., 174
Navin N., 81
Nawy T., 154
Nogueira F., 167
Nolan D., 43
Nolan M., 88
Nora E., 157

Okunola H., 38
Otazu G., 149
Oviedo H., 149

Packer R., 101
Paddison P.J., 188
Pagani M., 151
Pai D., 81
Paik R., 121
Papazoglu C., 75
Pardee T., 67
Park J.-H., 115
Pasternak S., 181
Paul A., 121
Perkowski D., 45
Peters S., 154
Peunova N., 115
Powers S., 77
Prasanth S.G., 53
Premsrirut P., 67

Qin H., 111
Quirk M., 131

Rajaram M., 77
Ramu U., 157
Ranade S., 131
Rappaport A., 67
Ratner A., 92
Rebbeck C., 60
Rebolini D., 174
Regulski M., 145, 174
Ren L., 181
Riggs M., 81
Roca X., 38
Rodgers L., 81
Rollins F., 60
Ronemus M., 81
Rooks M., 60
Rosenberg A., 88
Rosenfeld J., 185
Rossmann M., 53
Rozhkov N., 60

Salghetti S., 55
San Roman A., 161
Sarkar A., 167
Satoh-Nagasawa N., 161
Schalch T., 35
Schiapparelli L., 108
Schuck S., 50
Schwab R., 157
Schwertassek U., 94
Scuoppo C., 67
Sebat J., 143
Semighini C., 67
Serkhane J., 139
Sharma P., 108
Shaw S., 38
Shen R., 157
Shen W., 108
Sheu Y.-J., 53
Siddiqui K., 53
Silva J., 60
Silverman J., 185
Simon J., 67
Simorowski J., 157
Simorowski N., 119
Simpson D., 55
Sinha N., 145
Sinha R., 38
Siolas D., 60
Skowronski J., 90
Slotkin R.K., 157
Smear M., 131
Smith A.D., 185
Smith C., 60
Smith S., 35
Sordella R., 92
Sosluski D., 131
Sotirova V., 60
Spector D.L., 47
Spector M., \67
Spencer A., 43
Spiegel L., 174
Spooner W., 178, 181
Srivastava S., 90
Stein J., 181
Stein L., 178
Stenlund A., 50
Stillman B., 53
Sun S., 38
Sunwoo H., 47

Tai L.-H., 149
Tam O., 60
Tang C., 71
Taniguchi H., 121
Tansey W.P., 55
Tanurdzic M., 157
Thakar R., 47
Thirumala V., 108
Thomason J., 181
Timmermans M., 167
Tolia N., 35
Tonks N.K., 94
Tordo J., 115
Torres A., 92
Troge J., 81
Trotman L., 99
Tsigankov D., 129
Tully T., 145
Turner G., 147
Tzeng R.-Y., 108

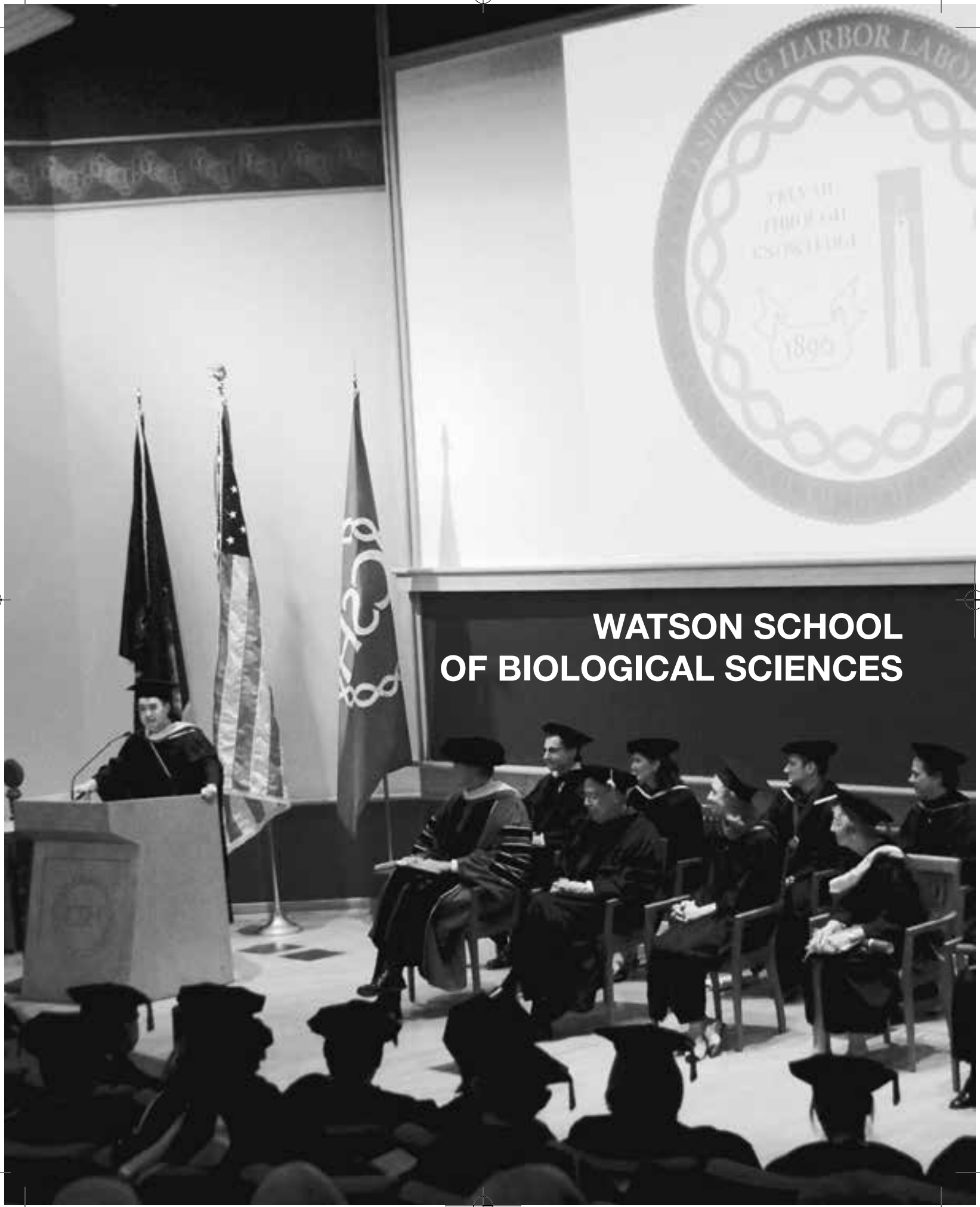
Uchida M., 131

Vagin V., 60
Valente D., 139
Van Aelst L., 101
van Buren P., 178, 181
Van Keuren-Jensen K., 108
Vasudevan D., 94
Vaughn M.W., 157
Vestin A., 75
Vidal M., 115
Wallace M., 38
Wang H., 139, 145
Wang J., 161
Wang L., 55
Wang X.W., 185
Wang Y., 151
Ware D., 181
Wei W., 136
Wen Q., 106
Wendel P., 53
Weng H.Y., 185
Whipple C., 161
Wigler M., 81
Williams J., 99, 154
Wilusz J., 47
Wu C., 139
Wu G., 178
Wu P., 121
Wu X., 121
Wu Y., 75

Xia S., 145
Xu C., 151
Xu N., 131
Xuan Z., 174
Xuan Z.Y., 185
Xue J., 157
Xue W., 67

Yamrom B., 81
Yan Y., 161
Yang H., 121
Yang M., 67
Yang Y., 60, 149
Yang Y.-T., 101
Yin P., 161
Yoon S., 143
Youens-Clark K., 181
Yuan M., 47

Zador A., 149
Zadrozny T., 161
Zaratiegui M., 157
Zariwala H.A., 131
Zender L., 67
Zha Z., 178
Zhan L., 88
Zhang C., 185
Zhang J., 77
Zhang L., 181
Zhang M.Q., 185
Zhang X.C., 94
Zhang Z., 38
Zhao R., 47
Zhao W., 178, 181
Zhao X.Y., 185
Zhao Z., 67
Zhong Y., 151
Zhou R., 60
Zuber J., 67



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WATSON SCHOOL OF BIOLOGICAL SCIENCES DEAN'S REPORT

Another year has flown by at the Watson School of Biological Sciences (WSBS) and we are now in our ninth year! After having served the School since its inception in 1999, I was appointed as the new Dean in the summer of 2007. Dr. Lilian Clark, our previous Dean, moved to London to become Executive Director of the Science Operations and Funding of Cancer Research U.K. Lilian had been with the School since its inception, rising from Assistant Dean to Associate Dean and, in 2004, to Dean. Her contributions to the School were many, from advising individual students on how to best approach their studies, to teaching and fund-raising, to successfully establishing the WSBS graduate program as a model for other graduate programs in the region. Lilian made sure that the School maintained its high standards, and matters were dealt with professionally and with great expertise. The School is deeply indebted to Lilian and wishes her all the best in her future endeavors.

Additional changes in the School's administration include the return to his research by the previous Director of Graduate Studies, Dr. William Tansey, and the departure of the School's Administrative Assistant, Eva Radeck.

As the new Dean, I plan to continue the School's commitment to excellence and to ensure that our students are prepared for the new era of quantitative biology. I have been associated with the Watson School in curriculum development, teaching, mentoring, and advising and directed our Undergraduate Research Program (URP) for 3 years. I plan to continue my active role as a member of the School's faculty as well as my research program.

Team Watson: (*Left to right*) Uwe Hilgert, Kim Geer, Leemor Joshua-Tor, Dawn Meehan, Alyson Kass-Eisler

To allow me to effectively and efficiently lead both the School and my research team, the School re-adopted its previous leadership structure consisting of an academic Dean and an administrative Assistant Dean. In October 2007, the School appointed Uwe Hilgert, Ph.D., as the School's Assistant Dean. Dr. Hilgert has been at CSHL's Dolan DNA Learning Center (DNALC) since 2000, where he developed courses and taught students, teachers, and college faculty to integrate bioinformatics investigations with bench work. More recently, Uwe served the DNALC as Assistant Director, managing its educational activities, including its satellite center, DNALC *West* in Lake Success. He also had a key role in preparing the groundwork for the DNALC's upcoming expansion into New York City. Also new to the School is Kim Geer. Kim came to us from a corporate environment and is the School's new Administrative Assistant.

In fall 2007, Dawn Meehan was promoted to Director of Admissions and Student Affairs. Dawn has been with the School since 2003 and continues to have a key role in the successful recruitment of outstanding students worldwide. Her efforts to increase the visibility of our program dramatically increased the number of students applying to the School. Dawn has also been instrumental in making sure that the needs of our students are met, so that they may devote their time and energy fully to their academic and research work.

Last but not least, Alyson Kass-Eisler, WSBS Postdoctoral Program Officer and Curriculum Coordinator since 2003, continues to skillfully organize our postdoctoral programs and graduate student curriculum. Her personal experiences as a Ph.D. student and postdoctoral fellow are extremely beneficial in ensuring that our graduate students and postdocs are provided with all of the necessary support to lay the foundation for the successful pursuit of their scientific careers. I am extremely grateful that Alyson continues to dedicate her expertise to the smooth and consistent flow of our programs.

Faculty Changes

In 2007, Adam Kepecs, Lloyd Trotman, and Doreen Ware joined the School as new faculty members. Dr. Kepecs came to CSHL in 2002 to join the laboratory of Zachary Mainen as a postdoctoral fellow and was appointed to Assistant Professor in February 2007. His research focuses on neuroscience, specifically the neurobiology of decision making, neural circuits and neuroeconomics. Assistant Professor Lloyd Trotman came to the Watson School in January 2007 from Memorial Sloan-Kettering Cancer Center where he conducted postdoctoral research. His research will focus on molecular mechanisms of tumor suppression, cancer treatment, and PTEN regulation. Our third new faculty member, Adjunct Assistant Professor Doreen Ware, has been at CSHL since 2001 and is a computational biologist with the USDA Agricultural Research Service. Her research focuses on comparative genomics in plants using bioinformatics and computational biology to study genome evolution and diversity. We are very excited to have Adam, Lloyd, and Doreen work with our students and look forward to their participation in other WSBS activities.

Faculty that left in 2007 include Dmitri (Mitya) Chklovskii, Tatsuya Hirano, Wolfgang Lukowitz, Michael Myers, Karel Svoboda, and Tim Tully. As faculty members, all six served the School and its students in various roles, including instructor, guest lecturer, rotation advisor, research mentor, examiner, and as members of the Thesis and/or Admissions Committees. Drs. Chklovskii, Svoboda, and Tully will continue to serve the School as adjunct faculty. We wish them well in their new endeavors.

The Fourth WSBS Graduation

Graduation is a highlight of the year and April 22, 2007 saw the Watson School's fourth graduation ceremony. The graduating class of 2007 was composed of Darren Burgess, Beth Chen, Catherine Cormier, Claudia Feierstein, Tomáš Hromádka, Elizabeth Murchison, and Gowan Tervo, who were awarded the Ph.D. degree, and Jonathan Kui, who was awarded a Master's degree. The graduation was once again a wonderful occasion where honorary degrees were bestowed on Drs. Sidney Brenner and Joseph Sambrook and on Mrs. Mary D. Lindsay. Dr. Brenner, who shared in the 2002 Nobel Prize in Physiology or Medicine, delivered the commencement address.

As with each graduation, we extended a special welcome to the family members and friends of our students. Among those, the parents of 2007 graduate Liz Murchison may have had the longest trip ever for a family to join a WSBS graduation: six weeks from Tasmania to New York, traveling by boat and train.

2007 WSBS Graduates

Student	Thesis advisor	Current position
Darren J. Burgess	Scott Lowe	With Dr. Alan Ashworth, Cancer Research U.K., London
Beth L. Chen	Dmitri Chklovskii	Currently exploring positions in San Francisco
Catherine Y. Cormier	Yuri Lazebnik	Scientific Liaison, Harvard Institute of Proteomics
Claudia E. Feierstein	Zachary Mainen	With Dr. Pierre-Marie Lledo, Institute Pasteur, Paris
Tomáš Hromádka	Anthony Zador	With Dr. Anthony Zador, CSHL
Jonathan Kui	Tim Tully	Clinical Molecular Laboratory Technician, Weill Medical College of Cornell University
Elizabeth Murchison	Gregory Hannon	With Dr. Gregory Hannon, CSHL
Dougal Gowan R. Tervo	Karel Svoboda	With Dr. Alla Karpova, Janelia Farm Research Institute

Teaching Award

At this year's graduation ceremony, Dr. Josh Dubnau was presented with the annual Winship Herr Faculty Teaching Award, named in honor of the School's founding Dean. Josh, who was lead instructor of

2007 Graduation: (*Left to right*) Lilian Clark, Jonathan Kui, James D. Watson, Elizabeth Murchison, Bruce Stillman, Beth L. Chen, Catherine Y. Cormier, Tomáš Hromádka, Claudia E. Feierstein, Darren J. Burgess, Dougal Gowan R. Tervo

THESIS DISSERTATION DEFENSES

ENTERING CLASS OF 2001

Claudia E. Feierstein, February 16, 2007

Representation of goals, actions, and outcomes in rat orbitofrontal cortex.

Thesis Examining Committee

Chair:	**Carlos D. Brody**
Research Mentor:	**Zachary Mainen**
Academic Mentor:	**Linda Van Aelst**
Committee Member:	**Anthony Zador**
Committee Member:	**Matthew Wilson,** *MIT*
External Examiner:	**Geoffrey Schoenbaum,** *University of Maryland School of Medicine*

Tomáš Hromádka, April 6, 2007

Representation of sounds in the auditory cortext of awake rats.

Thesis Examining Committee

Chair:	**Carlos D. Brody**
Research Mentor:	**Anthony Zador**
Academic Mentor:	**William Tansey**
Committee Member:	**Dmitri Chklovskii**
Committee Member:	**Zachary Mainen**
External Examiner:	**Richard Harris,** *Rutgers University*

Dougal Gowan R. Tervo, March 23, 2007

Development of molecular systems for the inactivation of synaptic transmission.

Thesis Examining Committee

Chair:	**Roberto Malinow**
Research Mentor:	**Karel Svoboda**
Academic Mentor:	**Carlos D. Brody**
Committee Member:	**Zachary Mainen**
Committee Member:	**Nathaniel Heintz,** *The Rockefeller University*
External Examiner:	**Thomas Sudhof,** *University of Texas, Southwest Medical Center*

ENTERING CLASS OF 2002

Darren J. Burgess, March 28, 2007

RNAi genetic screens for chemotherapy response modifier genes.

Thesis Examining Committee

Chair:	**Gregory Hannon**
Research Mentor:	**Scott Lowe**
Academic Mentor:	**Nicholas Tonks**
Committee Member:	**Josh Dubnau**
Committee Member:	**Senthil K. Muthuswamy**
External Examiner:	**Carol Prives,** *Columbia University*

Beth L. Chen, February 15, 2007

Neural network of C. elegans *from anatomy to behavior.*

Thesis Examining Committee

Chair:	**Alexei Koulakov**
Research Mentor:	**Dmitri Chklovskii**
Academic Mentor:	**Senthil K. Muthuswamy**
Committee Member:	**Carlos D. Brody**
Committee Member:	**Zachary Mainen**
External Examiner:	**Cori Bargmann,** *The Rockefeller University*

Elizabeth Murchison, April 9, 2007

Genetic studies of mammalian Dicer.

Thesis Examining Committee

Chair:	**Senthil K. Muthuswamy**
Research Mentor:	**Gregory Hannon**
Academic Mentor:	**John R. Inglis**
Committee Member:	**Alea A. Mills**
Committee Member:	**Janet Partridge,** *St. Jude's Children Hospital*
External Examiner:	**Richard Schultz,** *University of Pennsylvania*

THESIS DISSERTATION DEFENSES (*continued*)

ENTERING CLASS OF 2003

Hiroki Asari, July 2, 2007

Auditory system characterization.

Thesis Examining Committee

Chair:	**Carlos D. Brody**
Research Mentor:	**Anthony Zador**
Academic Mentor:	**Z. Josh Huang**
Committee Member:	**Alexei Koulakov**
Committee Member:	**Eero P. Simoncelli,** *New York University*
External Examiner:	**Barak Pearlmutter,** *National University of Ireland*

Rebecca A. Bish, December 13, 2007

A novel role for the ubiquitin-binding zinc finger domain in DNA repair.

Thesis Examining Committee

Chair:	**David L. Spector**
Research Mentor:	**Michael P. Myers**
Academic Mentor:	**Linda Van Aelst**
Committee Member:	**Scott Lowe**
Committee Member:	**Joshua LaBaer,** *Harvard University*
External Examiner:	**Rui-Ming Xu,** *New York University*

François Bolduc, October 2, 2007

Role of Drosophila *Fragile X mental retardation protein and the RNA interference pathway in* Drosophila *learning and memory.*

Thesis Examining Committee

Chair:	**Linda Van Aelst**
Research Mentor:	**Tim Tully**
Academic Mentor:	**Hollis Cline**
Committee Member:	**Yi Zhong**
Committee Member:	**Robert B. Darnell,** *The Rockefeller University*
External Examiner:	**Linda Restifo,** *University of Arizona*

Wei Wei, December 12, 2007

The interplay between amyloid-β and neural activity.

Thesis Examining Committee

Chair:	**Z. Josh Huang**
Research Mentor:	**Roberto Malinow**
Academic Mentor:	**Jan A. Witkowski**
Committee Member:	**Dimitri Chklovskii**
Committee Member:	**Yuri Lazebnik**
External Examiner:	**Sangram S. Sisodia,** *University of Chicago*

the *Specialized Disciplines* course in *Genetics*, was chosen by the students for his enthusiasm, excellence, and creativity in teaching, and here is some of what they had to say about the recipient:

Josh Dubnau

Josh went above and beyond expectations more than any other instructor we had. He was well prepared for every lecture and made his lectures interesting with great examples. He used humor and was a dynamic speaker, easily maintaining the class's attention and engaging the class in discussion and debate. Most importantly, he was approachable and readily available for extra help or just to discuss the material further. He clearly cared tremendously for the class and our understanding and appreciation of genetics and put in extra effort to make it fun and interesting and to make sure we understood the material.

Alumni Spotlights

Niraj Tolia, Ph.D., Entering Class 1999, received a B.Sc. from Imperial College of Science, Technology, and Medicine, London, U.K. Following his graduation from the Watson School in 2004, Dr. Tolia became a postdoctoral fellow in Leemor Joshua-Tor's laboratory. In December 2007, Dr. Tolia assumed a faculty position in the Department of Molecular Microbiology at Washington University School of Medicine in St. Louis.

Niraj Tolia

I remember my days as part of the first class entering the Watson School of Biological Sciences. It was an exhilarating and unique experience to be first in this exciting new model for graduate school and, by graduating within four years, to help prove the model a success. Eight years and several graduating classes later, I can say without a doubt that the Wat-

son School sets a new standard for higher education. This is a testament to the effort put forth by the faculty, administrators, and students to create an environment where students come first. Now, as an Assistant Professor of Molecular Microbiology at Washington University School of Medicine, I expand on my thesis research and study the neglected disease of Malaria. My journey would not have been what it is had it not been for the influence of the faculty at CSHL who were always willing to listen and offer constructive advice.

Alumni Highlights

The scientific achievements of WSBS students continue to be outstanding. As a consequence, WSBS graduates are thriving; they are successful in securing highly competitive awards and fellowships and

DOCTORAL THESIS RESEARCH

Student	Academic mentor	Research mentor	Thesis research
ENTERING CLASS OF 2001			
Claudia E. Feierstein *George A. and Marjorie H. Anderson Fellow* **Thesis Defense: February 2007**	Linda Van Aelst	Zachary Mainen	Odor coding and neural correlates of behavioral choice in the olfactory cortex.
Tomáš Hromádka *Engelhorn Scholar* **Thesis Defense: April 2007**	William Tansey	Anthony Zador	Stimulus optimization in the auditory cortex.
Dougal Gowan R. Tervo *George A. and Marjorie H. Anderson Fellow* *HHMI Predoctoral Fellow* **Thesis Defense: March 2007**	Carlos D. Brody	Karel Svoboda	An inducible and reversible lesion of the corticothalmic projection.
ENTERING CLASS OF 2002			
Allison L. Blum *Barbara McClintock Fellow*	Hollis Cline	Josh Dubnau	Genetic, behavioral, and anatomical characterization of Radish-dependent memory.
Darren J. Burgess *Engelhorn Scholar* **Thesis Defense: March 2007**	Nicholas Tonks	Scott Lowe	Mammalian RNAi genetic screens: Discovery and characterization of genes mediating the response to cancer therapy.
Beth L. Chen *Beckman Graduate Student* **Thesis Defense: February 2007**	Senthil K. Muthuswamy	Dmitri Chklovskii	Neuronal network of *C. elegans*: From anatomy to behavior.
Shu-Ling Chiu *Elisabeth Sloan Livingston Fellow*	Alea A. Mills	Hollis Cline	The role of insulin receptors in the development of neuronal structure and function.
Elizabeth Murchison *Engelhorn Scholar* **Thesis Defense: April 2007**	John R. Inglis	Gregory Hannon	The role of Dicer in mammalian development.
ENTERING CLASS OF 2003			
Hiroki Asari *Farish-Gerry Fellow* **Thesis Defense: July 2007**	Z. Josh Huang	Anthony Zador	Sparse overcomplete representation as a principle for computation in the brain.
Rebecca A. Bish *David H. Koch Fellow* **Thesis Defense: December 2007**	Linda Van Aelst	Michael Myers	A proteomics approach to the study of ubiquitylation.
François Bolduc *William R. Miller Fellow* **Thesis Defense: October 2007**	Hollis Cline	Tim Tully	Role of dFMR1 and the RNAi pathway in *Drosophila* learning and memory.
Monica Dus *Engelhorn Scholar*	John R. Inglis	Gregory Hannon	Characterization of the biological roles of the PIWI subfamily.
Angélique Girard *Florence Gould Fellow*	Jan A. Witkowski	Gregory Hannon	The role of the Piwi family and Piwi-associated small RNAs in mammalian spermatogenesis.
Christopher D. Harvey *David and Fanny Luke Fellow* **Thesis Defense: February 2008**	Adrian Krainer	Karel Svoboda	Visualization of MAPK activity in neurons.

DOCTORAL THESIS RESEARCH (*continued*)

Student	Academic mentor	Research mentor	Thesis research
Wei Wei *Leslie C. Quick Jr. Fellow* **Thesis Defense: December 2007**	Jan A. Witkowski	Roberto Malinow	Activity-dependent modulation of APP processing and Aβ production in rat hippocampal neurons.
ENTERING CLASS OF 2004			
Daniel H. Chitwood *George A. and Marjorie H. Anderson Fellow*	Alea A. Mills	Marja Timmermans	The contribution of small RNAs to positional signaling and the establishment of adaxial-abaxial polarity in leaves.
Galen A. Collins *Beckman Graduate Student*	Marja Timmermans	William Tansey	Role of ubiquitin ligases and activator destruction in transcription.
Oliver L. Fregoso *Seraph Foundation Fellow* *William Randolph Hearst Scholar*	Nicholas Tonks	Adrian R. Krainer	Proteomic analysis to elucidate the splicing and nonsplicing roles of SR proteins.
Keisha A. John *William Randolph Hearst Fellow and Scholar*	Josh Dubnau	Linda Van Aelst	Identification of the molecular determinants contributing to DOCK7's role in neuronal polarity.
Shraddha S. Pai *Charles A. Dana Fellow*	Anthony Zador	Carlos D. Brody	Determining the neuroanatomical loci and electrical correlates of duration discrimination in the rat.
David R. Simpson *Beckman Graduate Student*	Scott Lowe	William Tansey	Revealing insights into cancer biology with tumor-derived mutations in c-Myc.
ENTERING CLASS OF 2005			
Patrick M. Finigan *Beckman Graduate Student*	Senthil K. Muthuswamy	Bruce Stillman	ORC1 degradation in the cell cycle.
Amy Y. Leung *Beckman Graduate Student*	David L. Spector	William Tansey	Role of H2B ubiquitylation in chromatin localization.
Sarahjane M. Locke *George A. and Marjorie H. Anderson Fellow*	Josh Dubnau	Robert Martienssen	Histone H3 lysine 9 methylation and RNA processing in RNAi-mediated heterochromatin formation in *Schizosaccharomyces pombe.*
Hiroshi Makino *Elisabeth Sloan Livingston Fellow*	Hollis Cline	Roberto Malinow	Optical determination of the spatial distribution of experience-dependent bidirectional synaptic plasticity.
Katherine McJunkin *Robert and Teresa Lindsay Fellow*	Terri Grodzicker	Scott Lowe	Using negative-selection RNAi screens to identify novel treatment strategies for hepatocellular carcinoma.
Frederick D. Rollins *Cashin Fellow*	Jan A. Witkowski	Gregory Hannon	An RNAi screen for modifiers of cellular response to the targeted therapeutic Erlotinib.
Oliver Tam *Bristol-Myers Squibb Fellow*	David Jackson	Gregory Hannon	The role of RNAi machinery in oocyte maturation and embryonic development of the mouse.
Jeremy E. Wilusz *Beckman Graduate Student*	John R. Inglis	David L. Spector	Identification and functional characterization of large nuclear retained noncoding RNAs misregulated in breast cancer.
ENTERING CLASS OF 2006			
Yaniv Erlich *Goldberg-Lindsay Fellow* **Proposal Defense: February 2008**	John R. Inglis	Gregory Hannon	microRNA target identification by systematic sensor expression.
Eyal Gruntman *Elisabeth Sloan Livingston Fellow* **Proposal Defense: January 2008**	Nicholas Tonks	Robert Martienssen	DNA methylation mutants in *Arabidopsis thaliana* and their effect on whole-genome methylation patterns.
Colin Malone *Beckman Graduate Student* *NSF Graduate Research Fellow* **Proposal Defense: January 2008**	David J. Stewart	Gregory Hannon	Dissecting small RNA pathways in *Drosophila*.
Amy Rappaport *Barbara McClintock Fellow* **Proposal Defense: January 2008**	William Tansey	Scott Lowe	Identification and characterization of tumor suppressor genes in acute myeloid leukemia.
Claudio Scuoppo *Engelhorn Scholar* **Proposal Defense: February 2008**	Gregory Hannon	Scott Lowe	Identification of novel oncosuppressors through an in vivo RNAi screen in the Eμ-Myc model.

are moving into faculty positions much faster than, on average, other new Ph.D.s. By December 2007, 25 students had completed our Ph.D. program. We are extremely proud of the fact that two of our 2004 WSBS graduates have assumed tenure-track faculty positions within 8 (Niraj Tolia) and 7 (Ira Hall) years from matriculation—outstanding accomplishments and rare feats for most new Ph.D.s. Another 2004 WSBS graduate, Amy Caudy, holds an independent research position at the Lewis-Sigler Institute for Integrative Genomics at Princeton University. Dr. Rebecca Ewald, 2006 WSBS graduate, assumed a position in higher management with Roche Diagnostics in January 2008.

Although the short time span that has passed since most WSBS graduates received their degrees prohibits any meaningful statistics, the striking successes of our graduates clearly show that the program is living up to its promise and contributes extremely capable and productive individuals to science and academia worldwide.

Admissions 2007

The School received 243 applications for the 2007/2008 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 2007 entering class comprised Gregory Hannon (Chair), Josh Dubnau, Leemor Joshua-Tor, Adrian R. Krainer, Robert Lucito, David L. Spector, Nicholas Tonks, Linda Van Aelst, Anthony Zador, and William Tansey (ex officio)—a truly remarkable team!

Entering Class of 2007

On August 28, 2007, the Watson School opened its doors for the ninth time to welcome yet another new class. This time, an unprecedented 14 students joined the School: Megan Bodnar, Ralph Burgess, Joseph Calarco, Saya Ebbesen, Paloma Guzzardo, Kyle Honegger, Marek Kudla, Hassana Oyibo, Michael Pautler, Maria Pineda, Yevgeniy (Eugene) Plavskin, Joshua Sanders, Zhenxun Wang, and Petr Znamenskiy. Joseph, Paloma, and Marek previously participated in our summer undergraduate research program, and Hassana and Joshua had worked several months in the labs of CSHL researchers Robert Martienssen and Zachary Mainen, respectively.

Entering class of 2007 : (*Back, left to right*) Yevgeniy (Eugene) Plavskin, Zhenxun Wang, Kyle Honegger, Michael Pautler, Joseph (Joe) Calarco, Petr Znamenskiy, Ralph Burgess, Marek Kudla, Joshua Sanders. (*Front, left to right*) Paloma Guzzardo, Maria Pineda, Hassana Oyibo, Megan Bodnar, Saya Ebbesen

ENTERING CLASS OF 2007

Megan Bodnar, University of California, Santa Barbara
Starr Centennial Scholar
Academic Mentor: Nicholas Tonks

Ralph Burgess, University College London
Starr Centennial Scholar
Academic Mentor: Gregory Hannon

Joseph Calarco, University of Toronto
David Koch Fellow
Academic Mentor: Senthil K. Muthuswamy

Saya Ebbesen, Oberlin College
Starr Centennial Scholar
Academic Mentor: David J. Stewart

Paloma Guzzardo, University of Puerto Rico
Leslie C. Quick Jr. Fellow
Academic Mentor: Adrian R. Krainer

Kyle Honegger, Northwestern University
Crick-Clay Fellow
Academic Mentor: John R. Inglis

Marek Kudła, Warsaw University
George A. and Marjorie H. Anderson Fellow
Academic Mentor: David Jackson

Hassana Oyibo, State College at Farmingdale
Farish-Gerry Fellow
Academic Mentor: Hiro Furukawa

Michael Pautler, University of Guelph
William R. Miller Fellow
Academic Mentor: Robert Lucito

Maria Pineda, Barry University
Arnold and Mabel Beckman Graduate Student
Academic Mentor: Adrian R. Krainer

Yevgeniy (Eugene) Plavskin, Cornell University
Alfred Hershey Fellow
Academic Mentor: Jan A. Witkowski

Joshua Sanders, Stony Brook University
Edward and Martha Gerry Fellow
Academic Mentor: Bruce Stillman

Zhenxun Wang, University of Wisconsin, Madison
*A*STAR Fellow*
Academic Mentor: Alea A. Mills

Petr Znamenskiy, University of Glasgow
David and Fanny Luke Fellow
Academic Mentor: Terri Grodzicker

Reflecting CSHL's eclectic mix of nationalities, the entering class of 2007 is also quite international, with students hailing from Canada, Columbia, Poland, Puerto Rico, Russia, Singapore, the United Kingdom, and the United States.

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 as well as a research mentor. Entering students select, by mutual agreement, a member of the research or nonresearch faculty to serve as their academic mentor—a watchful guardian to look over and encourage the student through the sometimes trying process of their doctoral education. This program

Entering class of 2007. Nothing builds team spirit better than a shared boat ride...

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 Fall Term Curriculum

Our faculty continues to do an outstanding job developing and delivering the curriculum. As ever, the Curriculum Development and Integration Committee (CDIC) continues to carefully monitor and develop the curriculum. With the changes to the School's administration in the summer 2007, we also saw changes to the CDIC membership: Adrian R. Krainer replaced William Tansey as Chair, I replaced Lilian Clark, David L. Spector was replaced by David Jackson, and Z. Josh Huang and Nicholas Tonks were reappointed as members.

The fall 2007 *Scientific Reasoning and Logic* (*SRL*) core course, essentially unchanged from 2006, was taught by Gregory Hannon (Lead Instructor), Leemor Joshua-Tor, David Jackson, Scott Lowe, Robert Martienssen, and Glenn Turner, who replaced Anthony Zador.

The 2007 *Scientific Exposition and Ethics* (*SEE*) core course content remained essentially the same as it was in 2006, but the structure of the instructor team changed: It was reduced from three faculty to two—Jan A. Witkoswki as lead instructor and Alea A. Mills as instructor—and was supplemented by two postdoctoral fellows, Danielle Irvin and William Keyes, as teaching assistants, a format our students have taken to very well.

The content of the three *Specialized Disciplines in Biology* courses—*Genetics* and *Cellular Structure and Function*, and *Systems Neuroscience*—has largely remained the same. *Systems Neuroscience* was taught by its new lead instructor Anthony Zador, with guest lectures by CSHL faculty Drs. Brody, Cline, Kepecs, Koulakov, and Malinow. *Cellular Structure and Function* will this time be offered as an intensive, 1-week course in the spring of 2008 to accommodate co-instructor Dr. Raffaella Sordella, who went on maternity leave in fall 2007. Raffaella will teach this course with lead instructor Linda Van Aelst. Our courses continue to attract outstanding external guest instructors:

Course	Guest instructor	Affiliation
SRL	Thomas Clandinin	Assistant Professor, Neurobiology, Stanford University
	William C. Merrick	Professor, Biochemistry, Case Western Reserve University
	Charles J. Sherr	HHMI Investigator and Herrick Foundation Chair, Genetics and Tumor Cell Biology, St. Jude Children's Research Hospital
	Robert Tjian	HHMI Investigator and Professor, Molecular and Cell Biology, University of California, Berkeley
SEE	Melissa S. Anderson	Associate Professor and Director, Postsecondary Education Research Institute, University of Minnesota
	Katja Brose	Editor, *Neuron*, Cambridge, Massachusetts
	Maddy deLone	The Innocence Project, New York
	Nancy L. Jones	Associate Professor, Center for Bioethics & Human Dignity, Wake Forest University, North Carolina
	Philip Reilly	Chairman of the Board, Interleukin Genetics, Massachusetts
	Michael Rogers	Columnist, *Practical Futurist*, MSNBC, New York
SD-Genetics	Charalambos Kyriacou	Professor, Genetics, University of Leicester, U.K.
	Trudy MacKay	William Neal Reynolds Professor, Genetics, North Carolina State University, Raleigh

Recruiting Efforts

Recruitment for the graduate program's 2008 class and our URP 2008 was once again managed by Ms. Dawn Meehan, the School's Director for Admissions and Student Affairs. As in years past, Dawn traveled the length and breadth of the country representing CSHL and the WSBS. The table starting on the next page details recruitment fairs and conferences we have participated in, together with the names of

2007 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE

Event	Location	Date	WSBS representatives
Hunter College: Minority Access for Research Careers Program Visit and Information Session	CSHL	January 17	Dr. Leemor Joshua-Tor, Professor and Dean Dawn Meehan, Director of Admissions and Student Affairs Dr. William Tansey, Professor Dr. Anthony Zador, Professor
Wellesley College Women in Science Majors Fair	Wellesley College	February 14	Information sent for distribution
Ronald E. McNair Scholars Conference	University of Maryland, College Park	March 15–17	Dawn Meehan
Stony Brook University: Alliance for Graduate Education and the Professoriate Visit and Information Sessionr	CSHL	June 29	Dr. Josh Dubnau, Assistant Professor Dr. Leemor Joshua-Tor Dawn Meehan
The Protein Society 21st Annual Symposium Graduate Program Fair	Boston, Massachusetts	July 22	Angélique Girard, Graduate Student
Wabash College: Information Session	Wabash College	August 30	Galen Collins, Graduate Student
XVII Undergraduate Research Symposium NSF/ UMET Model Institutions for Excellence Project	Universidad Metropolitana, San Juan, Puerto Rico	September 14–16	Dawn Meehan
Hunter College: Minority Access for Research Careers Program Information Session	Hunter College	September 19	Dr. Leemor Joshua-Tor Dawn Meehan
MIT Career Fair	Massachusetts Institute of Technology	September 20	Amy Rappaport, Graduate Student
MIT Information Session	Massachusetts Institute of Technology	September 21	Amy Rappaport
Big 10+ Graduate School Expo	Purdue University	September 24	Colin Malone, Graduate Student
Washington DC Area Universities: Graduate School Fair	George Washington University	September 24	Amy Leung, Graduate Student
Pomona, Scripps, Harvey Mudd, Pitzer, and Claremont McKenna Colleges: Information Session	Pomona College	September 24	Galen Collins
Georgetown University: Information Session	Georgetown University	September 26	Amy Leung
The Johns Hopkins University: Information Session	The Johns Hopkins University	September 26	Jeremy Wilusz, Graduate Student
Notre Dame University: Information Session	Notre Dame University	September 26	Angélique Girard
Recruit in Canada Graduate School Fair	Toronto, Canada	September 30	Shraddha Pai, Graduate Student
Northwestern University: Information Session	Northwestern University	October 2	Dawn Meehan
Colgate University: Information Session	Colgate University	October 2	Dr. Robert Lucito, Assistant Professor
Cornell University: Information Session	Cornell University	October 2	Fred Rollins, Graduate Student Amy Rappaport
The Chicago Graduate and Professional School Fair	University of Illinois, Chicago	October 2	Dawn Meehan
Cornell University: Graduate and Professional School Day	Cornell University	October 3	Frederick Rollins Amy Rappaport
University of California, Berkeley: Information Session	University of California, Berkeley	October 9	Colin Malone
University of California, Berkeley: Graduate School Fair	University of California, Berkeley	October 10–11	Colin Malone
Society for Advancement of Chicanos and Native Americans in Science	Kansas City, Missouri	October 11–14	Dr. Adrian R. Krainer, Professor Dawn Meehan
University of Maryland, Baltimore County: Meyerhoff Scholarship Program Visit	University of Maryland, Baltimore County	October 12	Keisha John, Graduate Student
Emory University: Information Session	Emory University	October 15	Dr. Uwe Hilgert, Assistant Dean
California Institute of Technology: Career Fair	California Institute of Technology	October 17	David Simpson, Graduate Student
Atlanta Universities Consortium: Graduate and Professional School Information Day	Spellman College	October 17	Dr. Uwe Hilgert
University of California, Davis Graduate and Professional School Day	University of California, Davis	October 18	David Simpson
Emory University Graduate School Fair	Emory University	October 18	Dr. Uwe Hilgert
Haverford College: Information Session	Haverford College	October 22	Patrick Finigan, Graduate Student
Swarthmore College: Information Session	Swarthmore College	October 23	Patrick Finigan
University of Pennsylvania: Information Session	University of Pennsylvania	October 23	Patrick Finigan
Tulane University: Graduate School Fair	Tulane University	October 24	Dr. Uwe Hilgert
Washington University, St. Louis: Information Session	Washington University, St. Louis	October 24	Dr. Leemor Joshua-Tor
University of North Carolina, Chapel Hill: Information Session	University of North Carolina, Chapel Hill	October 25	Oliver Tam, Graduate Student
University of California, Irvine Graduate and Professional School Day	University of California, Irvine	October 29	Angélique Girard
Wesleyan University: Information Session	Wesleyan University	October 29	Dr. Uwe Hilgert
Sigma Xi Annual Conference and Research Symposium	Orlando, Florida	November 1–3	Dawn Meehan
California Forum for Diversity in Graduate Education: Graduate School Fair	University of California, Davis	November 3	Daniel Chitwood, Graduate Student
Yale University: Information Session	Yale University	November 5	Dr. Leemor Joshua-Tor
Dartmouth College: Information Session	Dartmouth College	November 6	Dr. Uwe Hilgert

2007 WATSON SCHOOL OF BIOLOGICAL SCIENCES RECRUITMENT SCHEDULE (*continued*)

Event	Location	Date	WSBS representatives
Princeton University: Information Session	Princeton University	November 8	Galen Collins
Annual Biomedical Research Conference for Minority Students	Austin, Texas	November 8–11	Keisha John Dr. Adrian R. Krainer Dawn Meehan
Princeton University: Graduate and Professional School Fair	Princeton University	November 9	Galen Collins
Harvard University: Information Session	Harvard University	November 15	Dr. David Jackson, Professor
Vassar College: Genetics and Bioinformatics Course Visit and Information Session	CSHL	November 16	Dawn Meehan Dr. Lincoln Stein, Professor Dr. Doreen Ware, Adjunct Assistant Professor
National Autonomous University of Mexico: Center for Genomic Sciences Undergraduate Program Visit	CSHL	November 26	Dr. Leemor Joshua-Tor Dawn Meehan Dr. Bruce Stillman, CSHL President Dr. Doreen Ware
Vanderbilt University: Information Session	Vanderbilt University	November 30	Dawn Meehan
Rice University: Information Session	Rice University	November 30	Information sent for presentation

faculty, students, and administrators who represented WSBS on these occasions. To further raise awareness of our programs, we also mailed more than 15,000 letters to colleges and universities in the United States and abroad.

Interinstitutional Academic Interactions

It is important to bear in mind that many of the graduate students who pursue their thesis research at CSHL are not in the WSBS graduate program. Indeed, the largest percentage of students are from Stony Brook University (SBU) via a program established between CSHL and Stony Brook more than 30 years ago. The 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. This year, the students listed in the text box below joined us.

Graduate Student Seminar Series

A very important element of the graduate student experience at CSHL is the weekly Graduate Student Seminar (GSS) series. These seminars are held from September through May and bring CSHL's diverse,

NEW STUDENTS FROM SHARED GRADUATE PROGRAMS

Student	CSHL research mentor	Affiliation and program
An-Yun Chang	Robert Martienssen	Stony Brook, Molecular and Cellular Biology
Xin Feng	Lincoln Stein	Stony Brook, Biomedical Engineering
Jong Jin Han	Robert Martienssen	Stony Brook, Molecular and Cellular Biology
Nihan Kara	Bruce Stillman	Stony Brook, Molecular and Cellular Biology
Matthew Lazarus	Z. Josh Huang	Stony Brook, Neuroscience
Deepika Vasudevan	Nicholas Tonks	Stony Brook, Molecular and Cellular Biology
Assaf Vestin	Alea A Mills	Stony Brook, Molecular and Cellular Biology
Bin Xue	Senthil K. Muthuswamy	Stony Brook, Molecular and Cellular Biology

multi-institutional graduate student community together to hear seminars from their colleagues. In the GSS, students present their research once a year, with two students presenting each week moderated by a fellow graduate student as seminar chair. In attendance are also two faculty mentors who rotate weekly.

The seminar series is open to the entire CSHL community and serves three important roles: (1) It gives students an opportunity to hone their oral presentation skills; (2) it provides students an opportunity to defend their research without the assistance of their research mentors such that they learn to stand on their own two feet as they will have to throughout their careers; and (3) it provides students in the audience an opportunity to ask questions within their own peer group, thus learning the important roles of audience participation for the advancement of research.

At the end of the evening, the attending faculty mentors provide presenting students a critique of their presentation. In addition, audience members anonymously complete seminar evaluation forms, which are given to the presenting students. In 2007, Josh Dubnau, Terri Grodzicker, Patrick Paddison, Arne Stenlund, Marja Timmermans, and Doreen Ware served as faculty mentors, bringing a breadth of knowledge and experience to the seminar series.

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 2007:

Postdoctoral Fellows

Nobuki Aono
Alfonso Apicella
Jannic Boehm
Jason Bohland
Deirdre Buckley
Ingrid Bureau
Vincenzo DePaola
Ana Domingos
Dominik Duelli
Rebecca Ewald
Tristan Fiedler
Andrea Gallavotti
Rita Gandhi
Supriya Gangadharan
Damien Garcia
Naria Gorovits
Michelle Hastings
Hongzhen He
Lin He
Shanta Hinton
Maarten Hoek
Joseph Jun
Prasanth Kumar Kannanganattu
Rotem Karni
Alla Karpova
Charles Kopec
Byeong Ha Lee
Hong Li
Ping-I Lin
Albert Mellick
Yuriy Mishchenko
Amitabh Mohanty
Tal Nawy
Itay Onn
Hong Li
Ping-I Lin
Dhruv Pant
Antonella Piccini
Xinying Ren
Fabiola Rivas
Jorge Santos DaSilva
Angshuman Sarkar
Mayil Shanmugam
Keishi Shintomi
Philip Smith
Takuya Takahashi
Niraj Tolia
Caroline van der Meijden
Yalin Wang
Linda Wilbrecht
Min Yu
Chih-Chi Yuan
Xiaoyue Zhao

Graduate Students

Darren Burgess
Cindy Chang
Beth Chen
Wonchang Choi
Catherine Cormier
Claudia Feierstein
Elvin Garcia
Adrienne Jones
Rebecca Kohnz
Shih-Chieh Lin
Sabrina Nuñez
Stephanie Shaw
Dougal (Gowan) Tervo
Ruei-Ying Tzeng
Quan Wen
Hong Zhao

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. In 2007, as the new Dean, I replaced Lilian Clark as Committee Chair. David L. Spector, the new Director of Research, joined the Committee as ex officio member, replacing Hollis Cline who left the Committee at the end of 2006. Assistant Dean Uwe Hilgert replaced

William Tansey, previously Director of Graduate Studies, as ex officio member of the Committee. As happens each year, there was also turnover among the student representatives. WSBS representative David Simpson was replaced by Eyal Gruntman. SBU representative Despina Siolas was replaced by Jane Lee-Osborne. We are thankful for their honest and thoughtful advice. I wish to also thank faculty members Terri Grodzicker, W. Richard McCombie, Alea A. Mills, Lincoln Stein, and Nicholas Tonks, all of whom willingly and diligently give their advice and oversight and, in addition, who continue to serve on the Executive Committee.

External Advisory Committee

An outstanding External Advisory Committee (EAC) guides the School. This EAC consists of Dr. Keith Yamamoto (Chair), Professor and Executive Vice Dean at the School of Medicine, University of California, San Francisco; Dr. Victor Corces, Professor, Department of Biology, Emory University, and Howard Hughes Medical Institute (HHMI) Professor; Dr. Gail Mandel, Senior Scientist, Vollum Institute, Oregon Health and Science University, and HMMI Investigator; Dr. Marguerite Mangin, Academic Programs Director and Senior Research Associate, The Rockefeller University; Dr. Barbara Meyer, Professor of Genetics and Development, University of California, Berkeley, and HHMI Investigator; and Dr. Frank Solomon, Professor, Department of Biology and Center for Cancer Research, Massachusetts Institute of Technology. The members of the Committee are noted for their leadership in graduate education in the biological sciences and are recognized leaders in their fields of research. Although the EAC provided significant input in 2007 to help guide the School through the restructuring of its leadership team, the Committee did not meet in person. An on-campus EAC meeting is currently being organized for July 2008.

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. David Baker, Professor of Biochemistry at the University of Washington and HHMI Investigator, was the 2007 Gavin Borden Fellow. His lecture "*Prediction and Design of Macromolecular Structures and Interactions*" was thoroughly enjoyed and evoked many questions from the audience. In addition, David shared his experiences as a scientist during dinner as well as at a more structured roundtable discussion with the students the following day.

Grants and Awards

Since 2002, WSBS has been the recipient of a Ruth L. Kirschstein National Research Service Award (also known as Predoctoral Training Grant) from the National Institute of General Medical Sciences (NIGMS) at the National Institutes of Health (NIH). Granted for 5 years, this award has funded up to five WSBS students annually. In 2006, the School submitted a 323-page competitive renewal application for this grant, requesting funding for six students. The evaluation process included a peer review and a site visit to the School by a five-person team led by NIH Training Program Director Dr. Marion Zatz in fall 2006. Subsequently, our application received an outstanding Priority Score and, on June 18, 2007, we received the fantastic news that the School had been granted an extension of its award for another 5 years.

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. In 2007, Colin Malone received a Graduate Research Fellowship from the National Science Foundation.

2007 WSBS STUDENT (CURRENT OR PREVIOUS) PUBLICATIONS

Aravin A.A., Sachidanandam R., **Girard A.**, Fejes-Toth K., and Hannon G.J. 2007. Developmentally regulated piRNA clusters implicate MILI in transposon control. *Science* **316:** 744–747.

Bish R.A. and Myers M.P. 2007. Werner helicase-interacting protein 1 binds polyubiquitin via its zinc finger domain. *J. Biol. Chem.* **282:** 23184–23193.

Brennecke J., Aravin A.A., Stark A., **Dus M.**, Kellis M., Sachidanandam R., and Hannon G.J. 2007. Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila. Cell* **128:** 1089–1103.

Brower-Toland B., Findley S.D., Jiang L., Liu L., Yin H., **Dus M.**, Zhou P., Elgin S.C., and Lin H. 2007. *Drosophila* PIWI associates with chromatin and interacts directly with HP1a. *Genes Dev.* **21:** 2300–2311.

Carmell M.A., **Girard A.**, van de Kant H.J.G., Bourc'his D., Bestor T.H., de Rooij D.G., and Hannon G.J. 2007. MIWI2 is essential for spermatogenesis and repression of transposons in the mouse male germline. *Dev. Cell* **12:** 503–514.

Chitwood D.H. and Timmermans M.C.P. 2007. Target mimics modulate miRNAs. *Nat. Genet.* **39:** 935–936.

Chitwood D.H., Guo M., Nogueira F.T.S., and Timmermans M.C.P. 2007. Establishing leaf polarity: The role of small RNAs and positional signals in the shoot apex. *Development* **134:** 813–823.

Dickins R.A., **McJunkin K.**, Hernando E., Premsrirut P.K., Krizhanovsky V., **Burgess D.**, Kim S.Y., Cordon-Cardo C., Zender L., Hannon G.J., and Lowe S.W. 2007. Tissue-specific and reversible RNA interference in transgenic mice. *Nat. Genet.* **39:** 914–921.

Harvey C.D. and Svoboda K. 2007. Locally dynamic synaptic learning rules in pyramidal neuron dendrites. *Nature* **450:** 1195–1200.

Houwing S., Kamminga L.M., Berezikov E., Cronembold D., **Girard A.**, van den Elst H., Filippov D.V., Blaser H., Raz E., Moens C.B., Plasterk R.H.A., Hannon G.J., Draper B.W., and Ketting R.F. 2007. A role for Piwi and piRNAs in germ cell maintenance and transposon silencing in zebrafish. *Cell* **129:** 69–82.

Hromádka T. and Zador A.M. 2007. Toward the mechanisms of auditory attention. *Hear. Res.* **229:** 180–185.

Huber D., Petreanu L., Ghitani N., Ranade S., **Hromádka T.**, Mainen Z., and Svoboda K. 2007. Sparse optical microstimulation in barrel cortex drives learned behaviour in freely moving mice. *Nature* **451:** 61–64.

Ibarra I., **Erlich Y.**, Muthuswamy S.K., Sachidanandam R., and Hannon G.J. 2007. A role for microRNAs in maintenance of mouse mammary epithelial progenitor cells. *Genes Dev.* **21:** 3238–3243.

Kim J., Inoue K., Ishii J., Vanti W.B., Voronov S.V., **Murchison E.**, Hannon G., and Abeliovich A. 2007. A microRNA feedback circuit in midbrain dopamine neurons. *Science* **317:** 1220–1224.

Kopec C.D., Real E., Kessels H.W., and Malinow R. 2007. GluR1 links structural and functional plasticity at excitatory synapses. *J. Neurosci.* **27:** 13706–13718.

Kopec C.D., Kessels H.W.H.G., Bush D.E.A., Cain C.K., LeDoux J.E., and Malinow R. 2007. A robust automated method to analyze rodent motion during fear conditioning. *Neuropharmacol.* **52:** 228–233.

Murchison E.P., Stein P., Xuan Z., Pan H., Zhang M.Q., Schultz R.M., and Hannon G.J. 2007. Critical roles for Dicer in the female germline. *Genes Dev.* **21:** 682–693.

Nogueira F.T.S., Madi S., **Chitwood D.H.**, Juarez M.T., and Timmermans M.C.P. 2007. Two small regulatory RNAs establish opposing fates of a developmental axis. *Genes Dev.* **21:** 750–755.

Tervo D. and Karpova A.Y. 2007. Rapidly inducible, genetically targeted inactivation of neural and synaptic activity in vivo. *Curr. Opin. Neurobiol.* **5:** 581–586.

Vaughn M.W., Tanurdžić M., **Lippman Z.**, Jiang H., Carrasquillo R., et al. 2007. Epigenetic natural variation in *Arabidopsis thaliana. PLoS Biol.* **5:** 1617–1629.

*Authors contributed equally to the work.

WSBS Family Events

Finally, I am pleased to announce that two WSBS students became parents and one was married: Beth Chen (entering class of 2002) and her husband Pavel welcomed a son, Nicholas; Eyal (entering class of 2006) and his wife Naomi Gruntman welcomed son Jonathan; and Wei Wei (entering class of 2003) married Peng Lui. Our congratulations and best wishes to all of them.

Leemor Joshua-Tor
Professor and Dean

SPRING CURRICULUM

Topics in Biology

ARRANGED BY **Alyson Kass-Eisler and Jan A. Witkowski**

FUNDED IN PART BY **The Daniel E. Koshland, Jr. Visiting Lectureship; The David Pall Visiting Lectureship; The Fairchild Martindale Visiting Lectureship; The Lucy and Mark Ptashne Visiting Lectureship; The Michel David-Weill Visiting Lectureship**

Each year, one or a team of invited instructors offer 7-day courses at the Banbury Conference Center to explore specialized topics outside the expertise of the Cold Spring Harbor Laboratory faculty. These courses include morning or evening lectures as well as afternoon sessions during which students read assigned papers. These intensive courses are modeled on the Cold Spring Harbor Laboratory Lecture Courses held each summer at the Banbury Conference Center. In spring 2007, there were two such courses: *Fundamental Concepts in Statistics* and *Evolution*.

Fundamental Concepts in Statistics

Attended by the entering classes of 2003 and 2004

INSTRUCTOR **Martina Bremmer,** Purdue University

GUEST LECTURERS **Madhu Mazumdar,** Weill Cornell Medical College

TEACHING FELLOWS **Cherie Ochsenfeld,** 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 students. It was accompanied by lab sections in which the students were

Statistics course participants: (*Back row, left to right*) François Bolduc, Christopher Harvey, Daniel Chitwood, Cherie Ochsenfeld, Martina Bremer, Gayla Olbricht, David Simpson, Galen Collins. (*Front row, left to right*) Monica Dus, Oliver Fregoso, Hiroki Asari, Keisha John, Wei Wei, Abhishek Chakraborty, Rebecca Bish, Cindy Chang, Shraddha Pai, Angélique Girard

instructed in 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 25–31, and was organized and largely taught by Martina Bremmer. This new WSBS course was highly anticipated by the students who, overall, thought it was one of the most useful and best organized courses they had taken.

Evolution

Attended by the entering classes of 2005 and 2006

INSTRUCTOR	**Nipam Patel,** University of California, Berkeley
GUEST LECTURERS	**Casey Bergman,** University of Manchester, U.K. **Rob DeSalle,** American Museum of Natural History **John Flynn,** American Museum of Natural History
TEACHING FELLOWS	**Philip Johnson,** University of California, Berkeley **Brian Kraatz,** University of California, Berkeley **Danielle Liubicich,** University of California, Berkeley

The field of evolutionary biology touches upon all other areas of biological sciences, because every form of life and every biological process represent an ongoing evolutionary "experiment." Our aim in this course was both to discuss our understanding of the mechanisms of evolution and to explore how evolutionary data can be used to further our understanding of various biological problems.

The course began with a discussion of the diversity of organisms that currently exist and our methods for understanding the evolutionary relationships between these organisms. We then went on to study how paleontological data are collected and used to understand the history of life on earth. We then examined how DNA sequence data can be used to understand the evolutionary history of organisms, genes, and genomes. Within this molecular and genetic framework, our focus shifted to the mechanisms of evolutionary change and how variation within populations leads to the evolution of new species. Finally,

Evolution course participants: (*Back row, left to right*) Frederick Rollins, Amy Rappaport, Colin Malone, Adrienne Jones, Claire Biot, Philip Johnson, Claudio Scuoppo, Eyal Gruntman, Hiroshi Makino, Jeremy Wilusz, Oliver Tam, Patrick Finigan, Yaniv Erlich. (*Front row, left to right*) Brian Kraatz, Danielle Liubicich, Amy Leung, Katherine McJunkin, Sarahjane Locke, Nipam Patel

we discussed how morphological changes are brought about through evolutionary changes in development and gene regulation. The hope was that this course would provide the student with a general overview of evolutionary biology and how to use evolutionary data to gain further insight into all manner of biological problems. The course ran from Sunday, February 18, through Saturday, February 24. As in previous years, the course was highly rated by all of the students.

Teaching Experience at the Dolan DNA Learning Center

DIRECTOR **David A. Micklos**

INSTRUCTORS **Greg Chin (Lead, high school)**
Amanda McBrien (Lead, middle school)
David Gundaker
Uwe Hilgert
Bruce Nash
Lauren Weidler

ADMINISTRATOR **Carolyn Reid**

As science plays an increasing part 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 one another 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, 12 faculty members served as rotation mentors, some mentoring more than one student.

ROTATION MENTORS

Hollis Cline
Josh Dubnau
Gregory Hannon
Scott Lowe
Robert Martienssen
Senthil K. Muthuswamy
Ravi Sachidanandam
Raffaella Sordella
Lincoln Stein
Glenn Turner
Linda Van Alest
Anthony Zador

FALL COURSE CURRICULUM

CORE COURSES

The Leslie C. Quick, Jr. Core Course on Scientific Reasoning and Logic

FUNDED IN PART BY **The Arnold and Mabel Beckman Foundation; The William Stamps Farish Lectureship**

INSTRUCTORS
Gregory Hannon (Lead)
David Jackson
Leemor Joshua-Tor
Scott Lowe
Robert Martienssen
Glenn Turner

GUEST LECTURERS
Josh Dubnau
Eric Enemark
Grigori Enikolopov
Hiro Furukawa
Adam Kepecs
Adrian R. Krainer
Senthil K. Muthuswamy
Patrick Paddison
Bruce Stillman
Anthony Zador

VISITING LECTURERS
Thomas Clandinin, Stanford University
William Merrick, Case Western Reserve University
Charles Sherr, St. Jude Children's Research Hospital
Robert Tjian, University of California, Berkeley

A fundamental aspect of earning the Ph.D. is training in the pursuit of knowledge. In this core course, which forms the heart of the curriculum, students (1) acquired a broad base of knowledge about the biological sciences, (2) learned the scientific method, and (3) learned how to think critically. This course consisted of six biweekly modules, each of which had a different theme. Each week, students read an assigned set of research articles and, at the end of the module, provided written answers to a problem set that guided them through several of the articles. Twice weekly, students attended lectures related to the week's topic that included concepts and fundamental information as well as experimental methods. During each week, the students met among themselves to discuss the assigned papers not covered by the problem set. Each week, students also spent one evening discussing the assigned articles with faculty. The last module, NIH Study Section, is unique in that the students assess proposed research that has yet to be completed, rather than looking back on science that has already been done. The module topics for this course were as follows:

Module 1	Gene Expression	Module 4	Signaling in Development
Module 2	Cell Proliferation and Cancer	Module 5	Neuroscience
Module 3	Macromolecular Structure and Function	Module 6	NIH Study Section

The Darrell Core Course on Scientific Exposition and Ethics

FUNDED IN PART BY **The Arnold and Mabel Beckman Foundation; The John P. and Rita M. Cleary Visiting Lectureship; The Seraph Foundation Visiting Lectureship; The Susan T. and Charles E. Harris Visiting Lectureship**

INSTRUCTORS **Jan A. Witkowski (Lead)**
Alea A. Mills

TEACHING ASSISTANTS **Danielle Irvine**
William Keyes

GUEST LECTURER **Walter Goldschmidts**

VISITING LECTURERS **Melissa Anderson,** University of Minnesota
Katja Brose, Editor, *Neuron*
Maddy deLeone, The Innocence Project
Nancy Jones, Center for Bioethics & Human Dignity
Philip Reilly, Interleukin Genetics
Michael Rogers, The Practical Futurist, MSNBC

This core course offered instruction in the fundamental elements of scientific exposition—writing skills and public speaking—and ethics. The ability to communicate effectively and to appreciate the intricacies of ethical issues are essential skills for biologists. Both subjects were taught in a series of example-based lectures and discussion groups. Writing skills included the fundamentals of modern scientific English and the organization and preparation of papers, research abstracts, and grant applications. Oral presentation skills were taught by instructors with different modes of presentation. Together with instructors, students critiqued formal seminar presentations at the Laboratory. Instruction and discussions about ethics included the ethical implications of biological discovery on society as well as the nature and boundaries of ethical behavior of scientists and their rights and responsibilities. A primary objective of the course was that students consider exposition and ethics an integral part of scientific research.

Research Topics

ORGANIZERS **Kimberley Geer**
Alyson Kass-Eisler

This core course provided students with an in-depth introduction to the fields of research that the Laboratory scientists investigate. Students and faculty attended a weekly Research Topics seminar, at which faculty members presented their current research topics and methods of investigation each Wednesday evening over dinner. The students learned how to approach important problems in biology. These seminars, together with the annual fall Laboratory In-House symposium, provided students with a basis for selecting laboratories in which to do rotations. The weekly speakers were:

Hollis Cline
Josh Dubnau
Grigori Enikolopov
Hiro Furukawa
Gregory Hannon
Z. Josh Huang
David Jackson
Leemor Joshua-Tor
Adam Kepecs
Alexei Koulakov
Adrian R. Krainer
Scott Lowe
Robert Lucito
Zachary Mainen
Robert Martienssen
W. Richard McCombie
Alea A. Mills
Vivek Mittal
Partha Mitra
Senthil K. Muthuswamy
Scott Powers
Jonathan Sebat
Raffaella Sordella
David L. Spector
Lincoln Stein
Arne Stenlund
Bruce Stillman
Marja Timmermans
Lloyd Trotman
Glenn Turner
Linda Van Aelst
Doreen Ware
Anthony Zador
Michael Q. Zhang
Yi Zhong

SPECIALIZED DISCIPLINES COURSES

This year, two of our Specialized Disciplines courses, *Systems Neuroscience* and *Genetics,* were held in the fall. The third course, *Cellular Structure and Function,* will be taught in Spring 2008.

Systems Neuroscience

FUNDED IN PART BY **The George W. Cutting Lectureship; The Klingenstein Lectureship**

INSTRUCTOR **Anthony Zador (Lead)**

GUEST INSTRUCTORS **Carlos D. Brody**
Hollis Cline
Adam Kepecs
Alexei Koulakov
Zachary Mainen
Roberto Malinow

This course introduced students to neuroscience, with a focus on learning and plasticity from its cellular basis, through development, to systems and behavior. Both experimental and theoretical viewpoints were explored. The course started with the basics of electrical signaling in neurons: ion channels, action potentials, and synaptic transmission. The cellular basis of learning including Hebb's postulates, LTP (long-term synaptic potentiation) was discussed. The course explored the consequences of synaptic learning rules by examining how experience shapes the wiring of the nervous system during development and investigated how such building blocks translate into whole-organism behavior. The course then examined classical conditioning and asked how changes in synaptic transmission could underlie such behavior. Associative learning computational models, of the learning process, were discussed as well.

From behaviors that focus on simple memories, the course turned to behaviors that require making perceptual decisions. To do this, it covered some basic concepts of perceptual neuroscience, such as neuronal "receptive fields," and used these to discuss current results and models of perceptual decision-making. Finally, the course turned to the learning of behaviors through reward and punishment, what is known as reinforcement learning. The course concluded with a discussion of the role of dopamine in reward and learning, the theory of reinforcement learning, and pathologies of reward-seeking behavior.

Genetics

FUNDED IN PART BY **The Edward H. and Martha F. Gerry Lectureship; The Pfizer Lectureship; The George B. Rathmann Lectureship; The Edward H. Gerry Visiting Lectureship**

INSTRUCTORS **Josh Dubnau (Lead)**
Lincoln Stein

VISITING LECTURERS **Bambos Kyriacou,** University of Leicester, U.K.
Trudy Mackay, North Carolina State University

In the past, "gene discovery" and association between gene and phenotype were accomplished in model organisms. Our understanding of human disease then was advanced by identification of human

orthologs associated with disease and by interventionist experiments using animal models of human disease. The completion of the human genome sequence and the remarkable advances in molecular biological techniques have initiated a paradigm shift in genetics. Associations between gene variants and disease now can be directly discovered in humans. Gene-to-phenotype functional associations can thus be discovered in humans as well as in model organisms. Causal mechanistic relationships between gene and phenotype can then be established using interventionist genetic experiments in animal models. This permits both a "vertical integration" to understand how molecular mechanisms influence functional output across various levels of biological organization and a "horizontal integration" to understand how genetic pathways have been conserved evolutionarily.

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, association studies, genome evolution, and genetic screens. How do gene mutations help to define biological processes? How are more complex traits genetically dissected into simpler (underlying) components? What concepts and techniques are used to organize genes into pathways and networks? How are genes mapped, cloned, and engineered to identify functional domains of proteins? What gene variation exists in natural populations? What are the functional consequences of gene variation? How is it detected? How are genomes organized and coordinately regulated? How can genomic information be catalogued, organized, and mined? These questions and concepts were fleshed out using examples from the literature.

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 approximately 140 postdoctoral fellows working in the laboratories of 40 principal investigators. Current fields of research expertise include genetics; molecular, cellular, and structural biology; neuroscience; cancer; plant biology; and genomics and bioinformatics. 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.

To enhance the postdoctoral experience at CSHL, an office for postdoctoral education (the Postdoctoral Program Office) was established within the WSBS to work closely with the postdoctoral fellows and the administration to coordinate and organize educational and career development activities. The Office provides support for current and arriving postdoctoral fellows as well as educational and career development programs. CSHL has an active Postdoctoral Association, whose purpose is to represent the interests of the CSHL postdoctoral community. In addition, the Postdoctoral Program Office also maintains a resource center containing career information and job and fellowship opportunities; it organizes workshops and career development seminars, job search forums, and grant-writing workshops and provides networking opportunities for postdoctoral fellows.

In 2007, there were significant changes to the WSBS administration. Dr. Lilian Clark, Dean since 2004, left CSHL to direct the Science Operations and Funding of Cancer Research U.K., in London. In July 2007, Dr. Leemor Joshua-Tor, a CSHL research faculty member since 1995, was appointed as Dean. Dr. Joshua-Tor has participated in curriculum development, teaching, mentoring, and admissions since the School's inception in 1999. In October 2007, Dr. Uwe Hilgert, who had been the Assistant Director of the Laboratory's Dolan DNA Learning Center, joined the WSBS as Assistant Dean. Drs. Joshua-Tor and Hilgert join Dr. Alyson Kass-Eisler, the Postdoctoral Program Officer, and Dr. Nicholas Tonks, the Scientific Director of the Postdoctoral Program, who have been members of the administration since 2003.

Postdoctoral fellows are encouraged to participate in many of the Laboratory's and Watson School's activities, as they are an integral part of the community. The Dean hosts "Dean's teas" with trainees who have recently arrived at CSHL, with the goal of helping fellows make the most of their postdoctoral experience here at CSHL. During these sessions, postdoctoral fellows are provided information on the services available through the Postdoctoral Program Office and given copies of the excellent books by Kathy Barker, "At the Bench: A Laboratory Navigator" and "At the Helm: A Laboratory Navigator," published by Cold Spring Harbor Laboratory Press. In addition, Laboratory President, Dr. Bruce Stillman, holds a "Town Hall" meeting specifically for postdoctoral fellows. This year's Town Hall meeting took place on December 18, 2007. Among the ideas that were discussed was the updating of the postdoctoral program Web site to enhance its usefulness as a networking tool. Alyson Kass-Eisler will be working with a small committee of postdocs to put together suggestions for the content and layout of this enhanced Web site.

In 2006, the Postdoctoral Program Office hosted a visit from Dr. Jing Li from the Merck Research Laboratories (MRL). CSHL has been a member of MRL's Ambassador Recruitment Program 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 visit, three of the Laboratory's trainees had their resumes submitted to the hiring managers at MRL and a postdoctoral fellow from Dr. Bruce Stillman's laboratory, Dr. Maarten Hoek, was hired as a Research Scientist in the Apoptosis Research Group. On December 7, 2007, Dr. Li returned to the School and 15 postdoctoral fellows discussed their career opportunities in one-on-one meetings with Dr. Li. We expect that a number of candidates will be considered for employment at MRL again this year.

CSHL's postdoctoral fellows have the opportunity to host an invited seminar speaker each year to participate in the Thursday Seminar series. This speaker is chosen based on nominations from the postdoctoral fel-

lows. The speaker for 2007 was Dr. Elizabeth Blackburn from the University of California, San Francisco. Dr. Blackburn is a wonderful speaker, scientist, and role model and has won numerous awards for her work including the 2006 Lasker Award. During her visit, Dr. Blackburn discussed new research developments in telomerase biology and in the responses of cells and organisms to perturbations in telomere maintenance. She also spent the day meeting with our postdoctoral fellows and faculty.

One of the nicest ways of exposing the Laboratory's trainees to different career opportunities is by having CSHL Alumni return to talk about their careers. This year, Dr. Alex Rai, a former graduate student with Dr. David Helfman, came to discuss his training with the American Association of Clinical Chemistry and his current position as an Assistant Attending at the Memorial Sloan-Kettering Cancer Center, Department of Clinical Laboratories.

In 2007, the Laboratory also hosted a special seminar by Dr. Joan Steitz from Yale University and the Howard Hughes Medical Institute entitled "*Beyond Bias and Barriers: Fulfilling the Potential of Women in Science*." This seminar was hosted by Dr. Michelle Hastings, a postdoctoral fellow in Adrian Krainer's laboratory. Dr. Joan Steitz is a member of the National Academy of Science and has been the recipient of many prestigious awards including the National Medal of Science and an honorary degree from our very own Watson School of Biological Sciences

Since 2003, all postdoctoral fellows and graduate students at the Laboratory are being enrolled in the Science Alliance of the New York Academy of Science (NYAS). The Science Alliance is a consortium of universities, teaching hospitals, and independent research facilities in the New York City metro area that have formed a partnership with NYAS. The aim of the Alliance is to provide career and professional development monitoring for postdoctoral fellows and graduate students in science and engineering, through a series of live events and a dedicated Web portal. In addition, the Science Alliance gives graduate students and postdoctoral fellows the opportunity to network with their peers across institutions and with key leaders in academia and industry. Lori Conlan, Director of the Science Alliance, NYAS, visited the Laboratory to discuss "*Navigating Your Career Path*" and followed the seminar with a one-on-one *curriculum vitae* writing clinic for postdoctoral fellows. Other Science Alliance programs this year included "*From Idea to IPO: The Technology Venture Course*," with Keynote Speaker David Anthony, Partner, 21 Ventures LLC; "*Getting Started as a Successful Grant Writer and Academician*" by Stephen W. Russell, D.V.M., Ph.D., Cofounder and Managing Member, Grant Writers' Seminars and Workshops, LLC; "*Professing Education*" with Keynote Speaker Sally Hoskins, City College of New York; and "Writing for Scientific Publication" with David C. Morrison, Ph.D.; GrantsCentral.com; Emma Hill, *Journal of Cell Biology*; Kalyani Narasimhan, Senior Editor at *Nature*; and John H.J. Petrini, Molecular Biology Program at Sloan-Kettering Medical Institute.

Another program, entitled "What Can You Be with a Ph.D.?" has since 1996 connected postdoctoral trainees with people who have discovered their own answer to that question. In 2007, the Science Alliance along with Columbia and New York Universities expanded the program to a 2-day Career Convention, which was attended and enjoyed by CSHL postdoctoral fellows.

In 2007, CSHL was extremely proud to be ranked in the Top 40 North American Institutes (#32), in The Scientist's annual survey of the "Best Places to Work: Postdoc." Inclusion in this elite group of institutions required positive feedback in the areas of Value of Postdoc Experience, Quality of Facilities and Infrastructure, Benefits, Remuneration and Compensation, and Family and Personal Life. We hope to be included once again in the 2008 survey, which is due out in March. In 2007, the WSBS also participated in the National Research Council's Assessment of Graduate Programs, which aims to provide a metric for comparing graduate programs in similar disciplines to each other. One component of this survey is the quality of the postdoctoral programs. We look forward to seeing the results of this survey, which will hopefully provide another measure of the success of our program and for ways in which it can be improved.

Finally, the most telling measure of our postdoctoral program's success is the ability of our postdoctoral fellows to secure permanent positions at the end of their training. In 2007, the Laboratory's departing postdoctoral fellows went on to positions at the Case Western Reserve University; *Cell* Press; The Chicago Medical School; Florida Institute of Technology; INMED/INSERM, Marseilles, France; International Centre for Genetic Engineering and Biotechnology, Trieste, Italy; Janelia Farm Research Campus; McKinsey & Co., Zurich Switzerland; Merck Research Laboratories; RIKEN, Japan; University of California, Berkeley; University of Illinois, Urbana-Champaign; and Washington University, St. Louis, to name just a few.

UNDERGRADUATE RESEARCH PROGRAM

Program Directors **David Jackson**
Lincoln Stein

Program Administrator **Dawn Meehan**

Every summer, approximately 25 undergraduates from around the world and across the country participate in the CSHL summer Undergraduate Research Program (URP) to acquire a taste of original research at the cutting edge of science. In 2007, 28 students participated in the URP, increasing the number of students who joined the program since its inception in 1959 to more than 700.

The fundamental objective of the program is to give students an opportunity to conduct first-rate research. Participants learn about scientific reasoning, laboratory methods, theoretical principles, and scientific communication. The specific objectives of the program are to (1) give college undergraduates a taste of conducting original research at the cutting edge of science, (2) encourage awareness of the physical and intellectual tools necessary for modern biological research, (3) foster an awareness of the major questions currently under investigation in the biomedical and life sciences, and (4) promote interactions with laboratory scientists through an immersion in the research environment.

During the 10-week program, URPs work with CSHL senior staff members on independent research projects, specifically in the areas of cancer biology, neuroscience, plant biology, cellular and molecular biology, genetics, macromolecular structure, and bioinformatics.

URPs work, live, eat, and play among CSHL scientists and have a very busy academic and social calendar for the summer. They attend lectures in the Goldberg Faculty Lecture Series from CSHL and outside faculty members including, in 2007, talks by Dr. Stanley Maloy, San Diego State University, and Dr. Peter Gergen, Stony Brook University. URPs also attend a seven-part Bioinformatics Workshop Series, where they learn how to identify patterns in DNA and protein sequences and how to interpret them. Lectures specifically designed for URPs were presented by CSHL faculty, including Dr. James Watson (10 Rules for Science) and Dr. Jan Witkowski (Scientific Ethics). 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 Plate Race and Scavenger Hunt, and the ever-famous URP versus PI volleyball match rounded out the engaging program. As in the previous 47 years, URPs have yet to beat the CSHL PI team!

2007 URPs wearing the T-shirt that won the URP T-Shirt Design Contest 2007: (*Front row, left to right*) Karla Claudio-Campos, Lindsey Courtney, Rachel Sachs, Sarah Timm, Cindy Puente, John Xue. (*Second row, left to right*) John Angiolillo, Edith Davis, Rebecca Krock, Christine Schenck, Christine Cho, Sarah Sansom, Erin Romberg, Kate Schmidt, Adrianna San Roman, Cherline Lee, Paul Wolski, Alison Spencer. (*Third row, left to right*) Matt Golub, Simon Quay, Ryon Graf, Vikram Agarwal, Andrew Pao, Emily Combs, Alexander Korman, Josh Silverman

At the beginning of the summer, each URP writes an abstract and presents a talk on his or her proposed research. Concluding the program in August, all URPs prepare a final report and present their results in 15-minute talks at the URP Symposium. The following 28 students, selected from over 800 applicants, took part in the 2007 program:

Vikram Agarwal, University of Texas, Austin
Advisor: **Dr. Lincoln Stein**
Sponsor: National Science Foundation and Department of Defense
Characterizing coverage and chromosomal rearrangement in the Watson genome.

Jack Angiolillo, Amherst College
Advisor: **Dr. Leemor Joshua-Tor**
Sponsor: National Science Foundation and Department of Defense
Attempting to crystallize and solve the structure for the ORC 2-3 subcomplex.

Christine Cho, Brown University
Advisor: **Dr. Josh Dubnau**
Sponsor: National Science Foundation and Department of Defense
microRNAs in memory: Characterization of miR276a expression.

Karla Claudio-Campos, University of Puerto Rico, Cayey
Advisor: **Dr. Gregory Hannon**
Sponsor: Howard Hughes Medical Institute
Devil facial tumor disease.

Emily Combs, Cornell University
Advisor: **Dr. Doreen Ware**
Sponsor: National Science Foundation and Department of Defense
Validation of noncanonical introns in rice and a yeast one-hybrid system in *Arabidopsis thaliana*.

Lindsey Courtney, Drury University
Advisor: **Dr. Wolfgang Lukowitz**
Sponsor: National Science Foundation and Department of Defense
Exploring localization and complementation of the MAPK pathway.

George Cutsail, University of Maryland, Baltimore County
Advisor: **Dr. Adrian R. Krainer**
Sponsor: Libby Fellowship
The role of MNK2 isoforms in SF2/ASF-mediated transformation.

Edith Davis, Wellesley College
Advisor: **Dr. Gregory Hannon**
Sponsor: Burroughs-Wellcome Fellowship
Identification of LATS-1 as a putative tumor suppressor gene.

Matt Golub, Stanford University
Advisor: **Dr. Partha P. Mitra**
Sponsor: National Science Foundation and Department of Defense
The red-eye flight: Memory of a lifetime.

Ryon Graf, University of California, Irvine
Advisor: **Dr. William Tansey**
Sponsor: Emanuel Ax Fellowship
How to screen for genes that stabilize the proto-oncogene Myc.

Alexander Korman, University of Texas, Austin
Advisor: **Dr. Yi Zhong**
Sponsor: Garfield Fellowship
Pi3-kinase Akt1 pathway in Alzheimers flies.

Rebecca Krock, Washington University, St. Louis
Advisor: **Dr. David L. Spector**
Sponsor: H. Bentley Glass Fellowship
Gene localization with respect to transcriptional status.

Cherline Lee, Tuskegee University
Advisor: **Dr. Bruce Stillman**
Sponsor: William Townsend Porter Foundation
A screen for genes that suppress the pol30-8 silencing defect.

Andrew Pao, The Johns Hopkins University
Advisor: **Dr. W. Richard McCombie**
Sponsor: Joan Redmond Read Fellowship
5′ ends of rice genome transcripts.

Cindy Puente, Hunter College
Advisor: **Dr. Alea A. Mills**
Sponsor: Hunter College Fellowship
Determining whether loss of heterozygozity of CHD5 is a prerequisite for tumorigenesis.

Simon Quay, Whitman College
Advisor: **Dr. Anthony Zador**
Sponsor: National Science Foundation and Department of Defense
The role of long-range callosal projections in the auditory cortex.

Erin Romberg, Oberlin College
Advisor: **Dr. Zachary Mainen**
Sponsor: National Science Foundation and Department of Defense
Uncertainty, decision-making, and orbitofrontal cortex.

Matthew Russell, University of California, San Diego
Advisor: **Dr. Senthil K. Muthuswamy**
Sponsor: Robert H.P. Olney Fellowship
Cell polarity and the initiation and progression of breast carcinoma.

Rachel Sachs, Princeton University
Advisor: **Dr. Alexei Koulakov**
Sponsor: National Science Foundation and Department of Defense
Application of the Watershed algorithm to spike sorting: Error analysis and improvement.

Adrianna San Roman, Williams College
Advisor: **Dr. David Jackson**
Sponsor: National Science Foundation and Department of Defense
Stop-and-go traffic: Regulating the gates of plasmodesmata.

Sarah Sansom, Ohio State University
Advisor: **Dr. Marja Timmermans**
Sponsor: Von Stade Fellowship
Understanding leaf polarity pathways.

Christine Schenck, Marist College
Advisor: **Dr. Robert Lucito**
Sponsor: William Shakespeare Fellowship
An investigation of histone modifications using ChIP-on-chip.

Kathryn Schmidt, Yale University
Advisor: **Dr. Linda Van Aelst**
Sponsor: Steamboat Foundation
The role of the X-linked mental retardation protein oligophrenin-1 in glutamate receptor signaling.

Josh Silverman, Duke University
Advisor: **Dr. Michael Q. Zhang**
Sponsor: C. Bliss Memorial Fund
Novel miRNAs: Just a few clicks away.

Alison Spencer, University of Rochester
Advisor: **Dr. Vivek Mittal**
Sponsor: Former URP Fellowship
Identification and preliminary characterization of the vascular endothelial growth factor receptor 2 expressing cell in murine bone marrow.

Sarah Timm, Dickinson College
Advisor: **Dr. Raffaella Sordella**
Sponsor: David and Fanny Luke Fellowship
Non-small-cell lung cancer: Animal models and cancer stem cells.

Paul Wolski, Cornell University
Advisor: **Dr. Hiro Furukawa**
Sponsor: Former URP Fellowship
Structural analysis of the NMDA receptor NR2D subunit ligand-binding core.

John Xue, Cambridge University
Advisor: **Dr. Robert Martienssen**
Sponsor: Former URP Fellowship
The regulatory roles of *ASYMMETRIC LEAVES1* and putative RNA-dependent RNA polymerases in *Arabidopsis*.

PARTNERS FOR THE FUTURE

Program Director **Yuri Lazebnik**

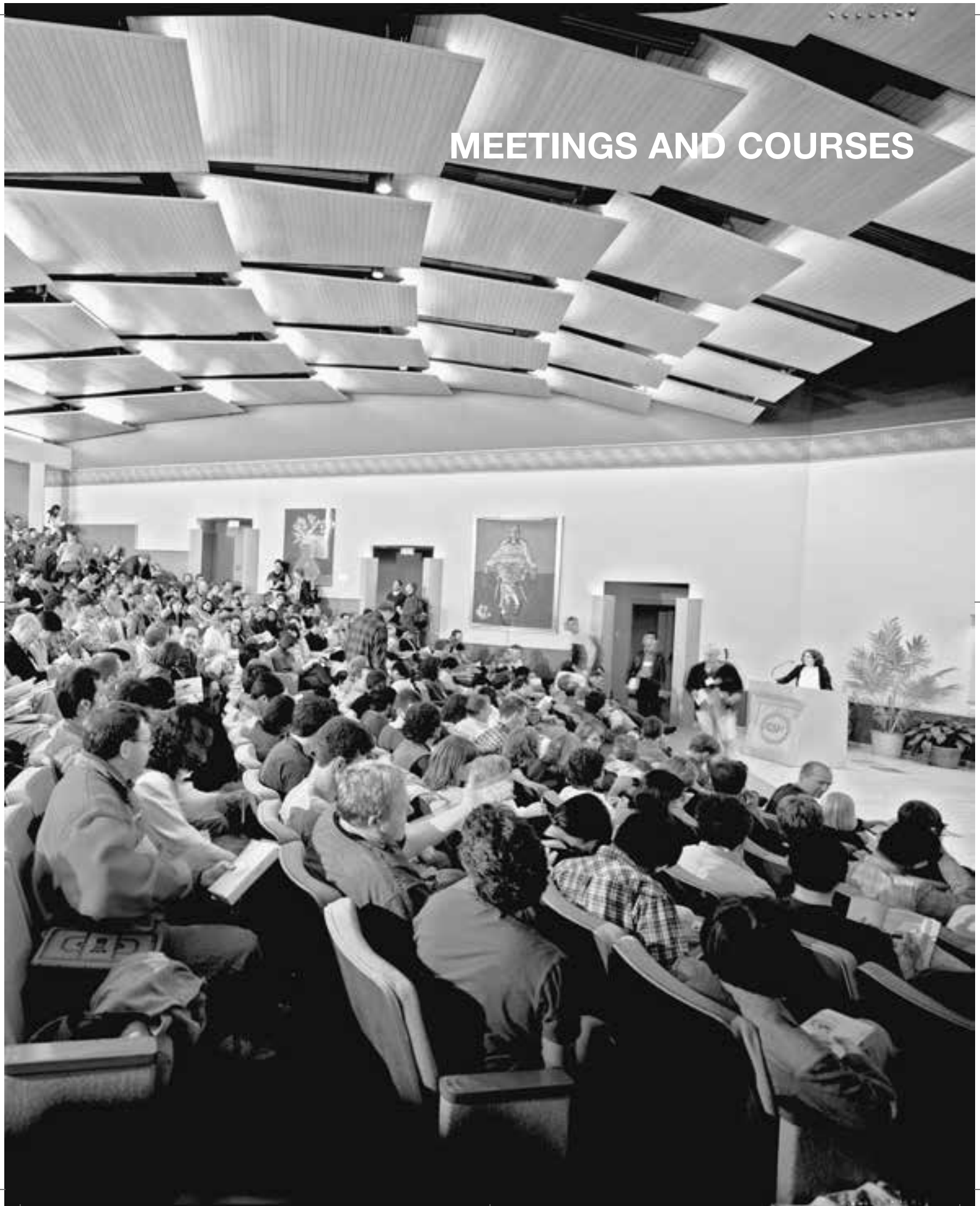
Program Administrator **Theresa Saia**

The Partners for the Future Program, established by Dr. James Watson in 1990, provides an opportunity for talented Long Island high school students to have hands-on experience in biomedical research at Cold Spring Harbor Laboratory. This highly competitive program is open to Long Island high school students in their junior year. Each high school science chairperson may nominate up to three students. The top candidates are interviewed by CSHL scientists and up to ten 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.

We are proud to report that Catherine Schlingheyde, a Senior at Oyster Bay High School and a 2006/2007 Partners for the Future participant, won fourth place among 1705 entrants in the 2007 national competition of the Intel Science Talent Search. Catherine conducted the research for her project on the workings of Argonaute, an important protein in the RNAi pathway, in the lab of Dr. Gregory Hannon and received a $25,000 fellowship along with her prize. The 2007–2008 Partners for the Future—chosen from among 23 nominations—are:

Partner	High school	CSHL mentor	Laboratory
Kristen DePre	Cold Spring Harbor High School	Keith Slotkin	Robert Martienssen
Madeleine Jensen	Huntington High School	Senthil K. Muthuswamy	Senthil K. Muthuswamy
Bobby Klein	Cold Spring Harbor High School	Gidon Felsen	Zachary Mainen
Katelyn Melgar	North Shore High School	Raffaella Sordella	Raffaella Sordella
Daniel Minkin	Portledge School	Lakshmi Muthuswamy	Michael Wigler
Damian Moskal	Glen Cove High School	Hsue-Cheng Chiang	Yi Zhong
Sweta Sanghavi	Floral Park Memorial High School	Yoselin Benitez Alfonso	David Jackson

2007/2008 Partners for the Future participants: (*Left to right*) Madeleine Jensen, Sweta Sanghavi, Katelyn Melgar, Kristen DePre, Bobby Klein, Damian Moskal, Daniel Minkin



MEETINGS AND COURSES

ACADEMIC AFFAIRS

The meetings and courses program at Cold Spring Harbor Laboratory communicates new discoveries, concepts, and methodologies to an international community of scientists. The program consists of advanced laboratory and lecture courses, short courses at the Woodbury Genome Center, and large meetings and biotechnology conferences that are held almost year round. More than 8000 scientists ranging from graduate students and postdoctoral fellows to senior faculty come from around the world to attend these events. A growing international program complements the main program of meetings and courses.

In 2007, 27 laboratory and lecture courses covered a diverse array of topics in molecular biology, neurobiology, structural studies, and bioinformatics. The primary aim of the courses is 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. New Banbury courses/workshops were started on Autism Spectrum Disorders and on Mechanisms of Alertness, Arousal, and Attention. The course on Molecular Embryology of the Mouse celebrated 25 successful years with a weekend reunion attended by many course alumni.

Instructors, course assistants, and course lecturers come from universities, medical schools, research institutes, and companies around the world to teach at Cold Spring Harbor Laboratory. Their excellence and dedication make the course program work so well. The full program of 2007 courses and instructors are listed on the following pages. We would especially like to thank Drs. Greg Bashaw, Mario De Bono, Abby Dernburg, Florian Engert, Tristan Fiedler, Janet Heasman, Mark Hubener, David Kleinfeld, Lincoln Stein, Scott Waddell, and Christopher Wylie who all retired this year after many years of service. We would also like to acknowledge the enormous teaching contributions of the late Dame Anne McClaren, who taught and lectured in many of our courses, in particular the mouse course at which she was a regular contributor almost every year since its inception in 1983.

Grants from a variety of sources support the courses. We were delighted to receive a significant increase in our core course program grant from the Howard Hughes Medical Institute. The courses are further supported by multiple awards from the National Institutes of Health (NIH) and the National Science Foundation (NSF). 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 24 meetings this year that brought together more than 7000 scientists from around the world to discuss their latest research. New meetings on Computational Cell Biology, Clinical Cardiovascular Genomics, and In Vivo Barriers to Gene Delivery, as well as a workshop on Honey Bee Biology and Genomics, were held. A prime feature of the meetings was that there were very few invited speakers. Meetings organizers selected talks from abstracts that were submitted. This format ensured that the latest findings were presented and that young scientists had the chance to describe their work.

Richard Treisman and Robert Tjian

The annual meetings on The Biology of Genomes and Retroviruses were oversubscribed, and many others attracted record attendances, including the meetings on Systems Biology: Global Regulation of Gene Expression, Eukaryotic mRNA Processing, and Neurobiology of *Drosophila*. Many of the 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 Patrons, Benefactors, Sponsors, Affiliates, and Contributors.

The Symposium—now in its 72nd year—continues to be the flagship conference of the meetings program. This year's meeting on *Clocks and Rhythms* addressed our current understanding of the structure and function of biological clocks and rhythms and featured an opening workshop, 72 talks, and 142 poster presentations. Opening night speakers included Joe Takahashi, Louis Ptáček, Amita Sehgal, and Ron Evans. Martin Raff presented the Reginald Harris lecture on "Intracellular timers in oligodendrocyte precursor cells." Charles Czeisler enlightened a mixed audience of scientists and lay friends and neighbors with his Dorcas Cummings lecture on "Work hours, sleep and safety: Physician heal thyself," and Michael Menaker ended the meeting with a masterful and eloquent summary.

The joint Cold Spring Harbor Laboratory/Wellcome Trust conference series held at the genome campus south of Cambridge, England, included meetings on Interactome Networks, Integrative Approaches to Brain Complexity, Functional Genomics and Systems Biology, and Pharmacogenomics. These conferences follow the Cold Spring Harbor Laboratory model in that the majority of talks are selected from the abstracts, and attracted 421 participants in total.

The success of the very large number of meetings and courses is also due to the skilled work of many Laboratory staff and faculty who contribute their expertise, efforts, and good humor to the program.

Terri Grodzicker
Assistant Director
Academic Affairs

David Stewart
Executive Director
Meetings and Courses Program

Bruce Stillman and Andrew Millar, Symposium speaker and former URP participant

Dame Anne McLaren and Gillian Bates at the 25th anniversary of the mouse course

Liz Murchison, Barbara Zane, and Andrew Mendelson

Terri Grozicker issuing the plate race rules

72ND COLD SPRING HARBOR SYMPOSIUM ON QUANTITATIVE BIOLOGY

Clocks and Rhythms

May 30–June 4 316 participants

ARRANGED BY **Bruce Stillman and David Stewart,** Cold Spring Harbor Laboratory

The history of research into circadian rhythms can be traced back to the French astronomer Jean Jacques Ortous de Mairan, who conducted experiments with plants grown in the dark in the early 1700s. His observations started a slow march joined by luminaries such as Carolus Linnaeus and Charles Darwin interested in whether and how animals and plants measured time. The descriptive era of circadian biology continued well into the latter half of the 20th century and was well summarized by the last Symposium held on the topic in 1960, in which the opening address by Erwin Bunning concluded that "thus far, however, such facts have not enabled us to draw far-reaching conclusions about the nature of the [biological] clock."

As with so many other disciplines in biology, the descriptive era has been revolutionized by the molecular era. Since the discovery and cloning of the first clock gene, *period*, more than 20 years ago, tremendous progress has been made about the nature of the clock and how it functions in a wide variety of different plants and animals. Research previously limited to describing "the hands of the clock" has been enormously successful in recent years in describing the inner anatomy and mechanism of the clock in individual cells and in the whole organism.

Many in the chronobiology community are now, more than ever, attempting to place the molecular and cellular details of the oscillatory machinery in the broader context of cellular physiology—for example, the cell cycle—or organismal behaviors—for example, sleep and circadian behavior, and how these may in turn be affected by the environment and/or disease. It is noteworthy that Christopher William Hufeland wrote in his 1796 treatise *The Art of Prolonging Life* "The period of twenty-four hours formed by the regular revolution of our Earth...is apparent in all diseases...it is, as it were, the unit of our natural chronology."

Our decision to focus the 72nd Symposium on circadian and related rhythms reflects the tremendous advances molecular and cellular approaches have yielded thus far, a decision taken in part thanks to an open letter from 18 prominent scientists in the field. The Symposium was arranged to cover a range of themes associated with biological clocks and rhythms, ranging in scale from the molecular to the whole organism, and addressed how aberrant function of the clock may have a causative role in diverse human diseases and conditions. The Symposium provided a unique synthesis of the exciting progress being made in the field of chronobiology not only for the Symposia attendees, but also for a wider global audience via interviews freely available on the World Wide Web and through the Symposium proceedings.

U. Schibler, B. Stillman

In organizing this Symposium with considerable help from Terri Grodzicker, we relied on the assistance of Steve Kay, Steve McKnight, and Ueli Schibler, in particular, for suggestions for speakers. We also wish to thank the first evening speakers, Joe Takahashi, Louis Ptáček, Amita Sehgal, and Ron Evans, for providing an overview of the areas to be covered. This year's Reginald Harris Lecture was delivered by Martin Raff on intracellular timers in oligodendrocyte precursor cells. We particularly wish to thank Michael Menaker—one of three participants along with Patricia DeCoursey and "Woody" Hastings, who attended both 1960 and 2007

Symposia—for delivering a masterful and eloquent summary of the current state of the field, and to Charles Czeisler who enlightened a mixed audience of scientists and lay friends and neighbors with his Dorcas Cummings lecture on "Work hours, sleep, and safety: Physician heal thyself."

This Symposium was attended by 316 scientists from more than 20 countries, and the program included an opening introductory workshop, 72 oral presentations, and 142 poster presentations. Essential funds to run this meeting were obtained from the National Institute of Neurological Disorders and Stroke, a branch of the National Institutes of Health. In addition, financial help from the corporate benefactors, sponsors, affiliates, and contributors of our meetings program is essential for these Symposia to remain a success and we are most grateful for their continued support. *Corporate Patrons* include Pfizer Inc. *Corporate Benefactors* include Amgen Inc.; GlaxoSmithKline; Merck Research Laboratories; and Novartis Institutes for BioMedical Research. *Corporate Sponsors* include Abbott Laboratories; Applied Biosystems; AstraZeneca; BioVentures, Inc.; Bristol-Myers Squibb Company; Diagnostic Products Corporation; Forest Laboratories, Inc.; GE Healthcare Bio-Sciences; Genentech, Inc.; Hoffman-La Roche Inc.; Johnson & Johnson Pharmaceutical Research & Development, LLC;

M. Rosbash, M. Raff

N. Kondo, P. DeCoursey

C. Czeisler, Dorcas Cummings Lecture

Kyowa Hakko Kogyo Co., Ltd.; New England BioLabs, Inc.; OSI Pharmaceuticals, Inc.; Pall Corporation; Sanofi-Aventis; and Schering-Plough Research Institute. *Plant Corporate Associates* include ArborGen; Monsanto Company; and Pioneer Hi-Bred International, Inc. *Corporate Affiliates* include Abcam Ltd.; Agilent Technologies; and USB Corporation. *Corporate Contributors* include Cell Signaling Technology; Epicentre Biotechnologies; Hybrigenics, SA; Illumina; inGenious Targeting Laboratory, Inc.; IRx Therapeutics, Inc.; and Millipore. *Foundations* include Albert B. Sabin Vaccine Institute, Inc. and Hudson-Alpha Institute for Biotechnology.

M. Macaluso, H. Wang

C. Mahoney, A. Nikiforov

C. Jackson, S. Li

C. Green, M. Hastings, S. Golden

Symposium interviews

PROGRAM

Symposium Workshop: Chronobiology Concepts and Nomenclature

Part I: Molecular Basis of Rhythms Generation
S. Mackey, *Texas A&M University*

Part II: Neurophysiology of Pacemaker Function
S. Kuhlman, *Cold Spring Harbor Laboratory*

Part III: Sleep and Rhythms
J. Duffy, *Harvard University*

Introduction
S. Golden, *Texas A&M University, College Station*

Clockworks I/Lifespan
Chairperson: M. Young, *HHMI/The Rockefeller University, New York*

Neuroanatomy and Circuits
Chairperson: A. Sehgal, *HHMI/University of Pennsylvania, Philadelphia*

Clockworks II and Proliferation
Chairperson: C. Green, *University of Virginia, Charlottesville*

Reginald B. Harris Lecture: Intracellular Timers in Oligodendrocyte Precursor Cells
M. Raff, *MRC Laboratory for Molecular Cell Biology and Cell Biology Unit, University College London*

Development and Aging
Chairperson: D. Bell-Pedersen, *Texas A&M University, College Station*

Systems Approaches
Chairperson: U. Schibler, *University of Geneva, Switzerland*

Human Clocks and Rhythms
Chairperson: S. McKnight, *University of Texas Southwestern Medical Center, Dallas*

Dorcas Cumming Lecture: Work Hours, Sleep, and Safety: Physician Heal Thyself
C. Czeisler, *Brigham and Women's Hospital*

Posttranscriptional and Posttranslational Mechanisms
Chairperson: M. Rosbash, *HHMI/Brandeis University, Waltham, Massachusetts*

Seasons and Mood
Chairperson: P. DeCoursey, *University of South Carolina, Columbia*

Models
Chairperson: M. Zatz, *Journal of Biological Rhythms, Bethesda, Maryland*

Genetics of Rhythms
Chairperson: R. Silver, *Columbia University, New York*

Entrainment and Peripheral Clocks
Chairperson: J. Dunlap, *Dartmouth Medical School, Hanover, New Hampshire*

Summary
M. Menaker, *University of Virginia*

Group Photo

MEETINGS

Computational Cell Biology

March 6–9 162 participants

ARRANGED BY **Thomas Pollard,** Yale University
Les Loew, University of Connecticut Health Center
John Tyson, Virginia Polytechnic Institute & State University

The molecular pathways and networks that regulate many aspects of cell physiology are now known in great detail, and they are so complex as to defy reliable understanding by informal, intuitive reasoning. During the past 10 years, it has become clear that computer simulations based on realistic biophysical models of regulatory networks can be useful in rationalizing diverse published observations and in guiding new experimental studies. Success in this endeavor requires close collaboration among life scientists, mathematical modelers, and computer scientists. In March 2001, Les Loew and his colleagues at the University of Connecticut Health Center hosted the first multidisciplinary workshop on Computational Cell Biology at the Cranwell Center in Lenox, Massachusetts. The meeting was attended by an enthusiastic group of quantitative biochemists and cell biologists, modeling experts, and software developers. The first meeting featured oral presentations and poster sessions that highlighted the interplay between experiments and models and a unique afternoon session of software demonstrations. By popular demand, the UConn group hosted two more workshops, in 2003 and 2005. At the third meeting, a leadership group was commissioned to find a more permanent "home" for the meeting, with Cold Spring Harbor Lab identified as the first place to try. When approached with this idea, David Stewart immediately gave his full support and scheduled the first Computational Cell Biology Meeting at Cold Spring Harbor in March 2007.

T. Pollard, L. Loew, J. Tyson

The 2007 meeting was organized by John Tyson, Les Loew, and Tom Pollard, assisted by a distinguished panel of session chairs. The format of the original workshops was maintained, including the popular software demos, along with an emphasis on the most mature areas of the field (calcium signaling, protein signaling networks, cytoskeleton and motility, development, bacterial physiology, cell growth, division, and death). For oral presentations, the Lenox meetings had relied on invited talks by established leaders in the field. For the CSH meeting, a decision was made to choose most oral presentations from abstracts submitted by registered participants. For such a young, evolving field of science, this was a risky decision, but it proved to be a great success. Many young graduate students and postdocs were given a chance to showcase their work before an interdisciplinary audience, and they were successful in illustrating the power of a combined experimental-theoretical attack on outstanding problems in cell biology. The quality of the poster session was also extremely high.

The meeting opened with a Keynote Address by Adam Arkin, who described his group's novel achievements in engineering the molecular regulatory networks of bacteria to desirable ends, such as identifying, attacking, and killing tumor cells. These themes were taken up by young investigators (You, Emonet, Weinberger, and Shou) who are building designer microorganisms.

Filling in at the last minute for a hospitalized Sydney Brenner, Tom Pollard gave the second Keynote Address on cell motility and cell septation. The associated session included talks by Les Loew, who is modeling Pollard's ideas about actin dynamics; Julie Theriot, who is collaborating with Alex Mogilner on cell shape and movement; and four young researchers (Civelekoglu, Needleman, Vavylonis, and Sun), who are working on the mechanics of cell division. Protein signaling networks were covered by

S. Sreenath, C. Athale

senior lecturers (Kholodenko and Sorkin) and junior scientists (Albert, Bakal, Bromberg, and Kinkhabwala). Calcium signaling was covered by senior lecturers (Augustine, Ehrlich, and Smith) and by a talented young African-American (Sherry-Ann Brown). Development was covered by senior lecturers (Reinitz and Shvartsman) and junior scientists (Umulis and Papatsenko). The cell cycle session featured young investigators (Guang Yao on the G_1-to-S transition in mammalian cells, and Joe Pomerening, on the role of positive feedback in regulating mitosis in embryos and somatic cells) and established investigators (Frank Uhlmann and Bela Novak on exit from mitosis in budding yeast). The meeting ended on a somber note (cell death), featuring beautiful work on p53 signaling (Galit Lahav and John Wagner) and caspase activation (John Albeck).

The uniformly high quality of the science reported at this meeting and the cooperative spirit of the experimentalists, theoreticians, and computer scientists in attendance confirmed the decision of the Cold Spring Harbor Meetings staff to welcome Computational Cell Biology into their regular meeting schedule. The meeting will continue on its biennial schedule (March, 2009) for the indefinite future. Quantitative measurements and realistic mathematical modeling are here to stay in molecular cell biology, and this CSHL meeting promises to be a flagship venue for reporting the best developing work in the field.

The meeting was well supported by a grant from the National Institutes of Health, through the National Technology Center on Networks and Pathways at the University of Connecticut Health Center. This support is sufficient for at least one more meeting on a similar scale.

PROGRAM

Keynote Address: Signaling, Uncertainty, and Design of Natural and Artificial Cellular Systems
A. Arkin, *University of California, Berkeley*

Microbial Physiology
Chairpersons: A. Arkin, *University of California, Berkeley;* F. Arnold, *California Institute of Technology, Pasadena*

Motility and Mitosis
Chairpersons: A. Mogilner, *University of California, Davis;* J. Theriot, *Stanford University School of Medicine, California*

Development
Chairpersons: S. Shvartsman, *Princeton University, New Jersey;* J. Reinitz, *Stony Brook University, New York*

Keynote Address: The Comprehensive and the Comprehensible
S. Brenner, *Salk Institute of Biological Studies*

Cell Signaling I
Chairperson: R. Iyengar, *Mount Sinai School of Medicine, New York*

Cell Signaling II
Chairperson: B. Kholodenko, *Thomas Jefferson University, Philadelphia, Pennsylvania*

Calcium Signaling
Chairpersons: G. Dupont, *Université Libre de Bruxelles, Belgium;* B. Ehrlich, *Yale University School of Medicine, New Haven, Connecticut*

Software Presentations
Chairpersons: R.D. Phair, *Integrative Bioinformatics, Rockville, Maryland;* P. Mendes, *Virginia Bioinformatics Institute, Blacksburg*

Cell Division Cycle
Chairperson: B. Novak, *Budapest University of Technology & Economics, Hungary*

DNA Damage and Cell Death
Chairperson: Y. Lazebnik, *Cold Spring Harbor Laboratory*

A. Cowan, J. Wagner

C. Fall, I. Moraru

Plant Genomes

March 15–18 137 participants

ARRANGED BY **Steven Briggs,** University of California, San Francisco
Gloria Coruzzi, New York University
Michael Snyder, Yale University

The theme of this meeting "From Sequence to Functional Networks" was meant to highlight how systems biology and network approaches have brought insights into genome function. The expertise of the three coorganizers spanned the three major areas of the meeting: Plant Systems Biology (Coruzzi), Genomics and Proteomics Technology (Snyder), and Translational Genomics (Briggs). A special organizational feature of the meeting was to include talks from nonplant model systems in order to stimulate cross-talk between plant and animal systems. This included inviting nonplant speakers as keynotes and as lead speakers in most of the six sessions.

As systems and network approaches require advances in technology development and use, the meeting also included a session on New Technologies (Computational and Experimental) and a workshop on Resources lead by Michael Snyder and Joe Ecker. The keynote talks spanned the three meeting themes: Transcriptional networks (J. Chory); Use of proteomic technologies to biological discoveries (J. Yates); and Small RNA silencing networks (D. Balcombe). The talks by nonplant speakers spanned these three broad themes as well: Developmental networks (S. Kim, Stanford; Aging in humans); and TF-DNA and the Second genetic code (T. Hughes, Toronto); Proteomics and metabolomics (M. Gerstein; Yale; Yeast protein networks), and Comparative genomics (K. Frazer; Perlegen Sciences; SNPs and complex trait networks in mice).

A memorable highlight of the exchange between plant and animal systems included discussion of Kim's talk on aging in humans, in which Ian Sussex observed that as plants age vertically, they might be more suitable for aging studies, where technical issues of comparing organs from individuals with different genotypes would not be an issue. Advances in monitoring RNA and metabolites at the tissue and cellular levels were highlighted in talks on gene networks in specific cell types (root, trichome, guard cells),

S. Briggs, J. Dangl, G. Coruzzi, D. Baulcombe

and even spanned to ionomics where a new method ICP-MS was used to understand the tissue localization of iron in plant seeds. Understanding gene network function was extended by comparative genomic approaches in the important new area of natural variation. The New Technologies session included bioinformatic and systems biology tools, including VirtualPlant, the Redundome, and Botany Array. There was a high level of discussion and interaction at the meeting across species and systems, both during the sessions and in informal interactions. The feedback on the meeting was that it was an excellent mix of genomics and technology advancement and also an excellent opportunity for the plant genome community to interact with the leaders of genomics and systems approaches in other model organisms.

This meeting was funded in part by the National Science Foundation.

PROGRAM

Keynote Speakers
J. Chory, *Salk Institute for Biological Studies, La Jolla, California*
J.R. Yates, *Scripps Research Institute, La Jolla, California*

Networks I: Development
Chairperson: J. Chory, *Salk Institute for Biological Studies, La Jolla, California*

Comparative Genomics
Chairperson: K. Frazer, *Perlegen Sciences, Mountain View, California*

Networks II: Response to Environment
Chairperson: J. Dangl, *University of North Carolina, Chapel Hill*

Proteomics and Metabolomics
Chairperson: M.L. Guerinot, *Dartmouth College, Hanover, New Hampshire*

New Technologies
Chairperson: K. Birnbaum, *New York University, New York*

Workshop: Resources
J. Ecker, *Salk Institute for Biological Studies, La Jolla, California*
M. Snyder, *Yale University, New Haven, Connecticut*

Keynote Speaker
D. Baulcombe, *Sainsbury Laboratory, John Innes Institute, Norwich, U.K.*

Translational Genomics
Chairperson: J. Leach, *Colorado State University, Fort Collins*

M. Dunn, S. McDonald

J. Willams

J. Yates III

P. Rabinowicz, D. Jackson

Imaging Neurons and Neural Activity: New Methods, New Results

March 22–25 198 participants

ARRANGED BY **Atsushi Miyawaki,** Riken Brain Science Institute
Joshua Sanes, Harvard University
Karel Svoboda, Janelia Farms, HHMI

This was the second time this conference was held; the first was in March, 2005. It focused on imaging neuronal structure and function using a variety of advanced techniques, including a wide range of light microscopy techniques, electron microscopy approaches, and magnetic resonance imaging. The conference also explored emerging methodologies for manipulating neuronal function with a particular emphasis on genetics and chemistry. This unique forum was designed to stimulate future research studies in this important area and to promote interactions among researchers in diverse basic scientific areas relevant to this field.

Richard Axel delivered a fascinating Keynote Address on how he and his colleagues have used genetically encoded probes of neuronal structure and function to learn about olfactory processing in mice and *Drosophila*. Other talks were divided between those given by invited speakers and those chosen from openly submitted abstracts.

Progress in the field was summarized in thoughtful concluding remarks by Karel Svoboda. In all, this conference attracted 198 participants and 84 abstracts. Both oral and poster presentations appear to include a substantial amount of unpublished work on the frontier of neuronal imaging. Active interaction was achieved between neurobiologists and chemists as well as between graduate students or postdocs and established scientists. The general consensus of participants was that the meeting should be held again at Cold Spring Harbor in 2009.

This meeting was funded in part with contributions from the Corporate Sponsors.

K. Svoboda

J. Sanes

PROGRAM

Novel Photoactivation and Tagging Methods
Chairperson: R. Kramer, *Columbia University, New York*

Imaging of Neurons
Chairperson: H. Cline, *Cold Spring Harbor Laboratory*

Keynote Speaker
R. Axel, *HHMI/Columbia University*

Imaging of Neuronal Excitation and Signaling I
Chairperson: R. Friedrich, *Friedrich-Miescher-Institute, Basel, Switzerland*

Imaging of Neuronal Excitation and Signaling II
Chairperson: R. Yuste, *Columbia University, New York*

Novel Methods to Dissect Neural Circuits
Chairperson: D. Chklovskii, *Cold Spring Harbor Laboratory*

LM (Superresolution)/EM
Chairperson: J. Sanes, *Harvard University, Cambridge, Massachusetts*

R. Yu, R. Axel

Systems Biology: Global Regulation of Gene Expression

March 29–April 1 310 participants

ARRANGED BY **Julia Bailey-Serres,** University of California, Riverside
Nir Friedman, Hebrew University, Israel
Bing Ren, University of California, San Diego/Ludwig Institute for Cancer Research

Gene regulation coordinates complex metabolic and developmental programs of biological organisms. The elucidation of distinct mechanisms of gene control is essential to the understanding of biological processes from the single cell to organismal level. Recently, rapid progress has been made in applying systems biology approaches to studies of gene regulation, and this trend was clearly evident at this fifth meeting. The meeting was marked by a record number of participants in its series and the high quality of oral presentations and posters. The 3-day program captured recent advances in the knowledge of gene regulatory mechanisms and in methods for collection and evaluation of the complex data sets that capture the complexities of global gene regulation.

B. Ren, N. Friedman, J. Bailey-Serres

The opening session included a keynote presentation by a noted interdisciplinary researcher, Uri Alon, who teases intricate networks in cells down to simple terms with tools of theoretical physics and experimental biology. The subsequent oral sessions were structured to blend computational and experimental research that addresses key issues in transcriptional and posttranscriptional regulation of gene expression, including characterization of *cis*-regulatory elements in the genome, analysis of gene regulatory networks, quantitative study of the dynamic transcriptional process in living cells, and systematic investigation of protein/RNA interactions.

This year's meeting also featured a well-received session on the global regulation of gene expression by microRNA (miRNA). Several talks exposed the importance of miRNA and other small noncoding RNAs in regulation of gene expression at multiple levels, from modification of histone methylation status to destabilization of transcripts to inhibition of mRNA translation. A session devoted to epigenetic regulation of gene expression was another major new addition and the highlight of the meeting. Two short oral sessions (four speakers) on Friday and Saturday afternoons segued into a poster session. This year, a best-poster prize was established for the first time (thanks to the journal *Genome Research*) and was awarded to Itai Yanai (Harvard), who presented a study on comparative transcriptomics in *C. elegans* and *C. briggsae*. Two preworkshop meetings were held to benefit attendees who wished to become more familiar with either computational or experimental approaches in use to study global gene expression networks.

R. Kulkarni, L. Stubbs

This meeting was funded in part by the National Science Foundation.

PROGRAM

Opening Session: Overture
Chairperson: N. Friedman, *Hebrew University, Jerusalem, Israel*

Keynote Address: Simplicity in Biology
U. Alon, *Weizmann Institute of Science, Rehovot, Israel*

Genomic Regulatory Elements
Chairperson: D. Koller, *Stanford University, California*

Global Regulation by miRNA
Chairperson: S. Tenenbaum, *State University of New York at Albany*

Transcriptional Regulatory Networks
Chairperson: M. Eisen, *University of California, Berkeley*

Evolution of Transcriptional Networks
Chairperson: M. Bulyk, *Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts*

Dynamics—Gene Expression Analysis in Single Cells—Change Over Different Timescales—Variation in Expression in a Single Cell Type
Chairperson: J. Weissman, *HHMI/University of California, San Francisco*

Posttranscriptional Processes
Chairperson: J. Bailey-Serres, *University of California, Riverside*

Epigenetics and Transcription
Chairperson: B. Ren, *Ludwig Institute for Cancer Research, La Jolla, California*

M. Eisen, M. Llinas

C. Kuttler, F. Nielsen

C. Herrmann, J. Reid, S. Aerts, A. Adler

Synapses: From Molecules to Circuits and Behavior

April 18–22 148 participants

ARRANGED BY **Hollis Cline,** Cold Spring Harbor Laboratory
Richard L. Huganir, Johns Hopkins University School of Medicine/HHMI
Thomas Sudhof, University of Texas Southwestern Medical Center

The brain consists of a vast network of excitable cells (neurons) that conduct electrical impulses and communicate with each other via specialized junctions (synapses). Information is processed and stored in the nervous system through patterns of electrical activity and via changes in the strength and structure of synapses. All aspects of nervous system function, including perception, cognition, and action, depend on proper information processing by synapses. As master regulators of neuronal excitability and synaptic communication, ion channels and receptors lie at the heart of neurobiology. In recent years, the molecular and cell biological analysis of neuronal ion channels and receptors has revolutionized our understanding of the basic mechanisms that control electrical signaling and synaptic function in the nervous systems. The convergence of advances in biochemistry, molecular genetics, microscopic imaging, and electrophysiology has made synaptic biology one of the most exciting and rapidly growing in neuroscience. Increasingly, scientists are moving to in vivo systems to investigate the synaptic basis of behavior in living animals via the genetic engineering of channels, receptors, and other synaptic proteins in mice and other model organisms.

R. Huganir, H. Cline

The entire field is poised for further breakthroughs that will not only illuminate basic workings of the brain, but also shed light on neurological and psychiatric diseases that stem from abnormal neuronal excitability and synaptic dysfunction. Indeed, genetic association studies already point to genes for synaptic structure and function as being involved in neuropsychiatric illnesses, in particular autism. Future advances will be facilitated by cross-fertilization of ideas and technologies between scientists studying channels, receptors, and synapses at all levels, in diverse organisms and using different methodological approaches. To provide a forum that unites this exciting multidisciplinary area, the Cold Spring Harbor Laboratory meeting on "Channels, Receptors, and Synapses" was renamed "Synapses: From Molecules to Circuits and Behavior."

The third "Synapses" meeting was very successful, bringing together participants from the United States, Europe, and Asia in an atmosphere of social and scientific exchange. A wide range of topics were discussed, including Modulation of channel and receptor function, Synapse formation, Presynaptic function, Synaptic signaling mechanisms, Synaptic plasticity, Synaptic plasticity and devel-

L. Jan, K. Shen

R. Zucker, S. Sigrist

opment, Synaptic plasticity and behavior, as well as Diseases of the synapse. Compared to earlier meetings, the 2007 meeting emphasized the physiology and pathophysiology of synapses and the behavioral and systems implications of their functions. More than 50 attendants were selected to give oral presentations of their work and the majority of the rest presented posters. Outstanding plenary lectures were given by Lily Jan and Catherine Dulac.

The overall response to the meeting was extremely positive, cementing the reputation of this Cold Spring Harbor conference in the field. Attendees enjoyed the breadth of subjects covered and the chance to interact with investigators in related but distinct fields. The unusual opportunity for junior investigators (including postdocs and students) to present their own work was welcomed. However, at the same time, it became clear that the field is in a state of change, a state that will be supported by the changing nature of this meeting and will lead to more systems-oriented views. On the basis of its continuing success, the "Channels, Receptors, and Synapses" conference will take place again in two years (April 2009) with the same organizers.

This meeting was funded in part by the National Institute on Drug Abuse and the National Institute of Mental Health, branches of the National Institutes of Health.

PROGRAM

Channel, Transporter, and Receptor Function
Chairpersons: D. Bergles, *Johns Hopkins University School of Medicine, Baltimore, Maryland*; A. Triller, *INSERM, Paris, France*

Synaptic Function
Chairperson: C. McBain, *NICHD, National Institutes of Health, Baltimore, Maryland*

Synapse Structure and Function
Chairpersons: C. Aoki, *New York University, New York*; M. Ehlers, *Duke University Medical Center, Durham, North Carolina*

Synapse Development
Chairpersons: A. Ghosh, *University of California, San Diego;* Y. Jin, *University of Calfiornia, San Diego*

T. Sacktor, C. Powell

P. Shrestha, M. Nakajima

Neuronal Plasticity
Chairpersons: R. Nicoll, *University of California, San Francisco;* G. Turrigiano, *Brandeis University, Waltham, Massachusetts*

Keynote Speakers
C. Dulac, *HHMI/Harvard University*
L. Jan, *HHMI/University of California, San Francisco*

Synaptic Circuits
Chairpersons: W. Kristan, *University of California, San Diego;* G. Turner, *Cold Spring Harbor Laboratory*

Synaptic Plasticity and Behavior
Chairpersons: R. Hen, *Columbia University, New York;* M. Wilson, *Massachusetts Institute of Technology, Cambridge*

Diseases of Synapses and Circuits
Chairpersons: M. Greenberg, *Children's Hospital, Boston, Massachusetts;* J. Shen, *Brigham and Women's Hospital, Boston, Massachusetts*

S. Chatterjee, R. Chottekalapanda

The Ubiquitin Family

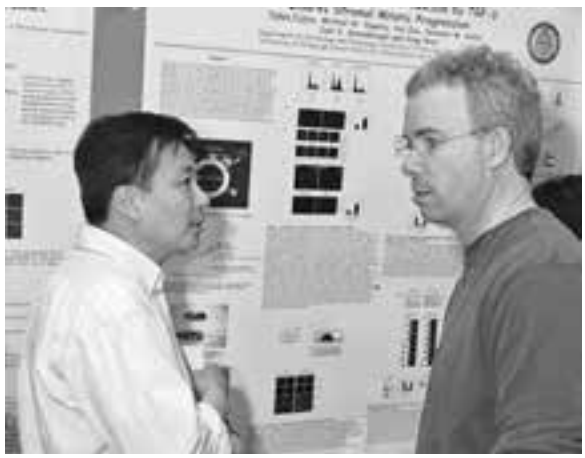
April 25–29 214 participants

ARRANGED BY **Joan Conaway,** Stowers Institute for Medical Research
Ray DeShaies, California Institute of Technology
J. Wade Harper, Harvard Medical School

This meeting represents the third Ubiquitin Family meeting following the successful inauguration of this series in 2003. It focused on ubiquitin and a group of structurally related "ubiquitin-like" proteins and their roles in regulation of various cellular processes. Major questions in the field include how specificity in ubiquitin and ubiquitin-like protein conjugation reactions is maintained and the molecular mechanisms that are used to control the specificity of ubiquitin chain linkages. These questions are being elegantly addressed using structural biology coupled with proteomics technology by an increasing number of investigators in the field. In addition, important advances continue to be made in understanding how ubiquitin and its family members contribute to the operation of diverse cellular pathways, and how these functions are perturbed in disease.

This year, the meeting attracted more than 212 scientists who engaged in lively discussions concerning the role of ubiquitin in protein turnover, the structure and mechanism of a large multisubunit protease called the proteasome that catalyzes the degradation of ubiquitinated proteins, and the involvement of ubiquitin in signaling and membrane trafficking. A wide array of systems—including yeast, *C. elegans*, and mammals—were used to probe the function of ubiquitin in diverse processes ranging from control of mitosis to processing of unfolded proteins to development of neuronal connectivity to control of mammalian circadian rhythms. In summary, this meeting has continued to be a unifying forum that helps us understand the ever-growing roles of ubiquitin and ubiquitin-like proteins in cellular physiology and disease.

This meeting was funded in part by the National Institutes of Health.



Y. Wan, W. Harper

R. DeShaies, M. Solomon

PROGRAM

UBL Conjugation, Removal, and Recognition I
Chairpersons: B. Schulman, *St. Jude Children's Research Hospital, Memphis, Tennessee;* D. Morgan, *University of California, San Francisco*

UBL Conjugation, Removal, and Recognition II
Chairpersons: F. Melchior, *University of Göttingen, Germany;* M. Peter, *ETH Hönggerberg, Zürich, Switzerland*

Nuclear Functions of UBL Proteins
Chairpersons: M. Dasso, *NICHD, National Institutes of Health, Bethesda, Maryland;* A. D'Andrea, *Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

Substrate Targeting and Degradation
Chairpersons: T. Baker, *HHMI/Massachusetts Institute of Technology, Cambridge;* C. Hill, *University of Utah, Salt Lake City*

Regulation of Intracellular Pathways
Chairpersons: K. Gould, *HHMI/Vanderbilt University, Nashville, Tennessee;* P. Jackson, *Genentech Inc., South San Francisco, California*

Quality Control
Chairpersons: K. Tanaka, *Tokyo Metropolitan Institute of Medical Science, Japan;* J. Frydman, *Stanford University, California*

UBLs in Signaling I
Chairpersons: P.P. DiFiore, *FIRC Institute of Molecular Oncology, Milano, Italy;* I. Dikic, *Goethe University Medical School, Frankfurt, Germany*

UBLs in Signaling II
Chairpersons: Z. Chen, *HHMI/University of Texas Southwestern Medical Center, Dallas;* A. Bonni, *Harvard Medical School, Boston, Massachusetts*

D. Germain-Desprez, M. Cohen

Y. Yashiroda, T. Kinsako

D. Skowyra, S. Appikonda

B. Buschhorn, D. Morgan

Telomeres and Telomerase

May 2–6 313 participants

ARRANGED BY **Joachim Linger,** Swiss Institute for Experimental Cancer Research
Vicki Lundblad, Salk Institute for Biological Studies
Dorothy Shippen, Texas A&M University

This conference consisted of eight sessions of talks and two poster sessions. As in 1999, 2001, 2003, and 2005, the format was to invite two chairs per session, who were a mix of established scientists in the field and younger scientists who had already made their mark by publishing as independent investigators. Many session chairs gave a scientific (12-min) presentation. The rest of the presentations (also 12-min) were chosen from submitted abstracts, allowing as many presentations as possible. These presentations were primarily by graduate students and postdoctoral fellows. Attendance exceeded 300 participants, a high fraction of whom presented the 149 posters and 87 talks.

The talks and posters covered all aspects of telomere and telomerase biology, including telomerase structure, enzymology, and regulation, telomere length regulation, protection and processing of chromosome ends, the consequences of telomere dysfunction, telomere dynamics in cancer, and telomerase-independent telomere maintenance.

The scientific content was very high throughout the conference in both the talks and posters. A large body of unpublished data was presented and extensively discussed in an open fashion. Formal and informal discussions were lively and informative. The conference was judged to be highly successful based on verbal and e-mail communications to the organizers. There is strong enthusiasm for another meeting on the same topic in 2009.

This meeting was funded in part by the National Institute on Aging, a branch of the National Institutes of Health.

E. Blackburn, C. Price, D. Shippen

PROGRAM

Telomerase Structure and Biochemistry
Chairpersons: T. Cech, *HHMI/University of Colorado, Boulder;* C. Autexier, *McGill University, Montreal, Canada*

End Resection and Protection
Chairpersons: J. Karlseder, *Salk Institute for Biological Studies, La Jolla, California;* E. Blackburn, *University of California, San Francisco*

Telomeres and DNA Damage Sensing
Chairpersons: T. de Lange, *The Rockefeller University, New York;* E. Gilson, *Ecole Normale Supérieure, Lyon, France*

Proteins Bound to Telomeric and Subtelomeric Chromatin
Chairpersons: F. Ishikawa, *Kyoto University, Japan;* D. Wuttke, *University of Colorado, Boulder*

Telomere Length Regulation
Chairpersons: D. Shore, *University of Geneva, Switzerland;* V. Zakian, *Princeton University, New Jersey*

Telomeres in Senescence, Proliferation, and Cancer
Chairpersons: J. Shay, *University of Texas Southwestern Medical Center, Dallas;* S. Artandi, *Stanford University School of Medicine, California*

Telomeres and Recombination
Chairpersons: R. Reddel, *Children's Medical Research Institute, Westmead, Australia;* M. McEachern, *University of Georgia, Athens*

Consequences of Telomere Dysfunction
Chairpersons: J.P. Cooper, *Cancer Research UK, London, United Kingdom;* C. Greider, *Johns Hopkins University, Baltimore, Maryland*

Wine and cheese at Airslie

S. Heyn, K. Lisaingo

S. Ahmed, C. Greider

T. Cech

P. Lansdorp, C. Harley

D. Shore, V. Zakian

Workshop on Honey Bee Genomics and Biology

May 6–8 92 participants

ARRANGED BY **Christine Elsik,** Texas A&M University
Gene Robinson, University of Illinois, Urbana-Champaign
George Weinstock, Baylor College of Medicine

This meeting was well attended by researchers representing 13 countries. The workshop began with keynote presentations by George Weinstock, William Gelbart, and Tom Seeley. They covered progress in improving the honey bee genome sequence, insights from the dozen *Drosophila* genome sequences, and an elegant behavioral dissection of colony decision-making in honey bee colonies, respectively. Scientific sessions included Population genetics; Evolution and comparative genomics; Neurobiology and behavior; Genomic technology and resources; and Caste, reproduction, aging, and immunity. There were 29 papers presented during the sessions. A lively poster session, containing 39 posters, was also held. A wrap-up session included an exploration of "what now" and "what next" with plans to hold subsequent meetings to build a strong honey bee genomics community.

This meeting was funded in part by Roche, Inc. and 454 Life Sciences, Inc.

G. Robinson, G. Weinstock

PROGRAM

Invited Speakers
G.M. Weinstock, *Baylor College of Medicine*
W.M. Gelbart, *Harvard University*
T. Seeley, *Cornell University*

Population Genetics
Chairpersons: R. Page, *Arizona State University, Tempe;* D. Smith, *University of Kansas, Lawrence*

Evolution and Comparative Genomics
Chairpersons: S. Richards, *Baylor College of Medicine, Houston, Texas;* H. Robertson, *University of Illinois, Urbana-Champaign*

Neurobiology and Behavior
Chairpersons: C. Grozinger, *North Carolina State University, Raleigh;* A. Mercer, *University of Otago, Dunedin, New Zealand*

Genomic Technology and Resources
Chairpersons: R. Maleszka, *Australian National University, Canberra;* D. Weaver, *Bee Weaver Apiaries Inc., Narasota, Texas*

Caste, Reproduction, Aging, and Immunity
Chairpersons: M. Beye, *Heinrich-Heine-Universität, Düsseldorf, Germany;* J. Evans, *USDA-ARS, Beltsville, Maryland*

Concluding Remarks
G.E. Robinson, *University of Illinois, Urbana-Champaign*

J. Anzola, B. Scharlaken

J. Gadau, G. Bloch

The Biology of Genomes

May 8–12 532 participants

ARRANGED BY **Michael Ashburner,** University of Cambridge
Kelly Frazer, Scripps Research Institute
Kerstin Lindblad-Toh, Broad Institute
Richard Wilson, Washington University

The annual Cold Spring Harbor Genome Sequencing and Biology meeting marked the 18th annual gathering of genome scientists in this setting. The past decade or more has seen remarkable progress in the mapping, sequencing, and annotation of the genomes of many "model organisms" and publication of finished and draft sequences of the human genome, in addition to several model organisms and hundreds of bacteria. Just over 500 people from around the world attended the meeting, with more than 300 abstracts presented describing a broad array of topics relating to the analysis of genomes from a number of different organisms.

K. Frazer, H. Sussman

The session topics included areas such as biological insights from Genetics and genomics of nonhuman species, High-throughput genetics and genomics, Cancer and functional genomics, Computational genomics, Population genomic variation, Genetics of complex traits, and Evolutionary genomics. There was considerable enthusiasm for the new resequencing technologies that are becoming available and the science that these technologies will enable. Dr. James Watson announced that his genome had been sequenced completely and would be released in the near future. The Friday afternoon keynote talks were delivered by Eddy Rubin and Thomas Hudson.

The ELSI (Ethical, Legal, and Social Implications) panel chaired by Francis Collins focused on ethical and social implications of research on natural selection in humans.

This meeting was funded in part by the National Human Genome Research Institute, a branch of the National Institutes of Health; Roche, Inc.; and Solexa, Inc.

R. Durbin, E. Lander

H. Peckham, F. De la Vega, M. Ashburner

PROGRAM

Genetics and Genomics of Nonhuman Species
Chairpersons: D. Kingsley, *HHMI/Stanford University, California;* G. Weinstock, *Baylor College of Medicine, Houston, Texas*

High-throughput Genomics and Genetics
Chairpersons: E. Mardis, *Washington University School of Medicine, St. Louis, Missouri;* M. Snyder, *Yale University, New Haven, Connecticut*

Cancer and Functional Genomics
Chairpersons: A. Feinberg, *Johns Hopkins University, Baltimore, Maryland;* M. Meyerson, *Dana-Farber Cancer Institute, Boston, Massachusetts*

Computational Genomics
Chairpersons: R. Durbin, *Sanger Institute, Hinxton, United Kingdom;* D. Haussler, *University of California, Santa Cruz*

ELSI Panel Discussion: Ethical and Social Implications of Research on Natural Selection in Humans
Moderator: F. Collins, M.D., Ph.D., *National Human Genome Research Institute*

Panelists: P. Sabeti, M.D., Ph.D., *Broad Institute, Massachusetts Institute of Technology*
S. Tishkoff, Ph.D., *Department of Biology, University of Maryland*
M. Cho, Ph.D., *Center for Biomedical Ethics, Stanford University*

Population Genomic Variation
Chairpersons: A. Clark, *Cornell University, Ithaca, New York;* P. Sabeti, *Broad Institute, Cambridge, Massachusetts*

Genetics of Complex Traits
Chairpersons: G. Abecasis, *University of Michigan, Ann Arbor;* P. Donnelly, *University of Oxford, United Kingdom*

Guest Speakers
E. Rubin, *Joint Genome Institute, Lawrence Berkeley National Laboratory*
T. Hudson, *Ontario Institute of Cancer Research*

Evolutionary Genomics
Chairpersons: H. Robertson, *University of Illinois, Urbana-Champaign;* J. Bennetzen, *University of Georgia, Athens*

W. Worley, J. Reecy, T. Hubbard

J. McPherson, E. Mardis

H. Basta, M. McClure, J. Lyles

C. Wade, C. Hitte

Phosphorylation, Signaling, and Disease

May 16–20 222 participants

ARRANGED BY **Sara Courtneidge,** The Burnham Institute
Ben Neel, Beth Israel Deaconess Medical Center
Nicholas Tonks, Cold Spring Harbor Laboratory

This seventh meeting brought together 222 scientists from the United States, Europe, the Far East, and the South Pacific. Specifically focused on tyrosine phosphorylation in prior years, we expanded this meeting to include the most current work on the structure, regulation, and function of all protein kinases and protein phosphatases in biology. The meeting began with two opening Keynote Addresses by Phil Cohen and Carol Greider.

The format of the meeting was designed around areas of biological interest, rather than according to particular enzymes or enzyme families. A major thrust within the pharmaceutical industry is to exploit signal transduction pathways as sources of targets for novel therapeutic strategies. Therefore, the program for this meeting addressed the role of protein phosphorylation in the regulation of signal transduction under normal and pathophysiological conditions.

The program included scientists from the United States, Europe, the Far East, and the South Pacific. There were 51 speakers selected to present their data in sessions that dealt with Receptor-proximal signaling; Physiology and disease; Cancer; Metabolic and stress signaling; Signaling pathways in survival and proliferation, and Model systems. The sessions were chaired by Deborah Morrison (National Cancer Institute, Frederick, Maryland), Anjana Rao (Center for Blood Research, Harvard Medical School, Boston), Gary Gilliland (HHMI/Brigham and Women's Hospital, Boston), John Blenis (Harvard Medical School, Boston), Roger Davis (HHMI/University of Massachusetts Medical School, Worcester), Pier-Paolo Pandolfi (Memorial Sloan-Kettering Cancer Center, New York), and Alexandra Newton (University of California, San Diego). A variety of systems were described with great progress reported in genetic and biochemical approaches to the characterization of physiological functions for protein phosphorylation.

The meeting was supported by grants from the National Cancer Institute, a branch of the National Institutes of Health, and with contributions from the Corporate Sponsors.

N. Tonks, A. Newton

P. Cohen, M. Arnone

PROGRAM

Keynote Speakers
P. Cohen, *University of Dundee*
C. Greider, *Johns Hopkins University School of Medicine*

Receptor-proximal Signaling I
Chairperson: D. Morrison, *National Cancer Institute, Frederick, Maryland*

Physiology and Disease
Chairperson: A. Rao, *Center for Blood Research, Harvard Medical School, Boston, Massachusetts*

Cancer
Chairperson: G. Gilliland, *HHMI/Brigham and Women's Hospital, Boston, Massachusetts*

Metabolic and Stress Signaling
Chairperson: J. Blenis, *Harvard Medical School, Boston, Massachusetts*

Signaling Pathways in Survival and Proliferation
Chairperson: R. Davis, *HHMI/University of Massachusetts Medical School, Worcester*

Model Systems
Chairperson: P.-P. Pandolfi, *Memorial Sloan-Kettering Cancer Center, New York*

Receptor-proximal Signaling II
Chairperson: A. Newton, *University of California, San Diego*

U. Guhe, J. Cho, S. Eminaga, J.-Z. Wu

B. Stronach, P. Marignani

K. Boyle, M. Hilger

J. Haley, S. Muthuswamy

Retroviruses

May 22–27 489 participants

ARRANGED BY **Thomas Hope,** Northwestern University
Ned Landau, New York University

This conference was centered on recent advances in HIV/AIDS and other human and nonhuman retroviruses. Keynote Speakers Joe Sodroski and Bart Haynes presented engaging talks: Joe Sodroski summarized the role of TRIM5α as an anti-HIV host factor. Dr. Sodroski identified TRIM5α as the major factor that restricted HIV-1 replication in old world monkeys. Human TRIM5α does not restrict HIV-1, but a point mutation that changes a single amino acid of the protein causes it to become a potent antiviral. This single amino acid of TRIM5α has allowed HIV-1 to infect and spread in the human population. At some point in human evolution, it is likely that individuals will be selected that have a mutation in TRIM5α that protects them and their offspring from becoming infected with HIV-1; however, how long that will take is not known. Dr. Haynes is the head of the NIH/Gates funded Center for HIV AIDS Vaccine Initiative (CHAVI). He reviewed the origins of the consortium and its current structure and operation as well as some of the findings that it has made to date. These include a genome-wide scan for human polymorphisms associated with resistance to AIDS. Two loci were identified, one of which was a gene for a class I major histocompatibility complex protein.

T. Hope, D. Krementsov

The more than 100 talks presented focused on all aspects of retrovirology. The host resistance factors TRIM5α and APOBEC3G were an area of notable research advances. The structure of the catalytic domain of APOBEC3G was presented and several groups presented their findings on its mechansim of inhibition. An unexpected role for APOBEC3G in blocking reverse transcription was detected. A fascinating study from Mike Emerman's group investigated the role of TRIM5α in preventing the infection of early humans about seven million years ago with a nonhuman primate retrovirus. A nonfunctional copy of the virus has been preserved in the chimpanzee genome. A fragment of the virus was "brought back to life" by putting it into a currently active retrovirus. The resulting virus was susceptible to inhibition by human TRIM5α, suggesting that this protein had protected early humans from becoming infected.

K. Jones, N. Landau

This was the first year of the annual Andy Kaplan prize that commemorates Dr. Kaplan, a prominent AIDS researcher who died last year. The prize was won by Sara Sawyer (Fred Hutchinson) who showed that TRIM5α is one of the most highly selected genes in the human genome. This gene is selected during evolution to have specific amino acid changes that allow TRIM5α to effectively restrict the replication of retroviruses.

Another cellular gene, *LEDG/F*, was found to interact with the HIV integrase protein to direct integration of the viral DNA into transcriptionally active sites in the genome. Several talks probed the structure–function of the protein and knockout cells were generated and found not to support efficient integration of the virus.

Several talks focused on the Vpr accessory proteins of HIV-1. Vpr was shown to interact with a specific cellular E3 ubiquitin ligase that has a role in DNA replication and cell cycle control. Because of the interaction, infected cells arrest in the G_2 phase of the cell cycle.

Other areas addressed included virus entry, assembly, reverse transcription, viral pathogenesis, and novel antivirals. Frank Kirchhoff (Ulm, Germany) reported on the development of a novel peptide inhibitor of HIV-1 entry. Interestingly, the peptide targets gp41 at its amino-terminal hydrophobic fusion peptide, a domain of the protein not previously thought of as amenable to drug targeting.

Funding for this meeting was provided by the CSHL Corporate Sponsor program.

PROGRAM

Entry
Chairpersons: L. Albritton, *University of Tennessee Health Science Center, Memphis;* J. Mak, *Burnet Institute, Melbourne, Australia*

Assembly I
Chairpersons: M. Johnson, *University of Missouri, Columbia;* K. Musier-Forsyth, *Ohio State University, Columbus*

Restriction Factors I (TRIM)
Chairpersons: C. Aiken, *Vanderbilt University, Nashville, Tennessee*; W. Johnson, *Harvard Medical School, Southborough, Massachusetts*

Keynote Speaker
B. Haynes, *Duke University Medical Center*

Assembly II
Chairpersons: J. Lingappa, *University of Washington, Seattle;* M. Resh, *Memorial Sloan-Kettering Cancer Center, New York*

Integration
Chairpersons: S. Chow, *University of California School of Medicine, Los Angeles;* A.M. Skalka, *Fox Chase Cancer Center, Philadelphia, Pennsylvania*

Keynote Speaker
J. Sodroski, *Dana-Farber Cancer Institute, Harvard Medical School*

RT/Antivirals
Chairpersons: M. Roth, *UMDNJ–Robert Wood Johnson Medical School, Piscataway, New Jersey;* G. Tachedjian, *Burnet Institute, Melbourne, Australia*

Pathogenesis
Chairpersons: J. Dudley, *University of Texas, Austin;* A. Cimarelli, *Ecole Normale Supérieure de Lyon, France*

Restriction Factors II (APOBEC)
Chairpersons: X-F. Yu, *Johns Hopkins University, Baltimore, Maryland;* V. Pathak, *National Cancer Institute, Frederick, Maryland*

Accessory Proteins
Chairpersons: P. Cannon, *Children's Hospital Los Angeles, California;* C. de Noronha, *Albany Medical College, New York*

Transcription/Processing/Export/Translation/Packaging
Chairpersons: A. Rice, *Baylor College of Medicine, Houston, Texas;* S. Carpenter, *Washington State University, Pullman*

R. Swanstrom, P. Jolicoeur

H. Taylor, L. Jones

S. Srivastava, S. Chakraborti

Yeast Cell Biology

August 15–19 272 participants

ARRANGED BY **Kerry Bloom,** University of North Carolina, Chapel Hill
Peter Pryciak, University of Massachusetts Medical School
Lois Weisman, University of Michigan

This conference was the 12th biannual international meeting devoted to diverse aspects of cell biology in yeast. This conference is unusual in that it encompasses a wide range of topics in cell biology but is focused on a simple eukaryotic organism, the budding yeast *Saccharomyces cerevisiae*, and includes other single-celled fungi such as *Candida albicans*, *Pichia pastoris*, and *Schizosaccharomyces pombe*. This year, research advances were presented in sessions organized around several major cell biological systems. New developments were presented in the areas of the actin and microtubule cytoskeletons, membrane trafficking, cell cycle and cell division, and signal transduction mechanisms. In addition, functional genomics, proteomics, and bioinformatics were featured prominently, with remarkable examples of using yeast to study genome-wide changes that accompany evolution.

K. Bloom

It has become increasingly clear during the last several years that cell biological systems do not operate in isolation but are instead highly coordinated, and the mechanisms of this coordination represent some of the most exciting research areas covered at the meeting. These include the organization of membranous organelles by the cytoskeleton, subcellular compartmentalization of cell cycle control proteins, regulation of cytoskeletal organization by signal transduction pathways, and compartmentation within the nucleus. Complementing these integrative topics were new computational models for complex molecular behavior, and new microscopic techniques for probing cell ultrastructure. The result is that studies of yeast are approaching an unprecedented degree of sophistication in the comprehensive, system-wide understanding of cellular function.

L. Weisman

By virtue of a common interest in one organism, instead of one topic in cell biology, the Yeast Cell Biology meeting continues to encourage extensive cross-fertilization of ideas, insights, and methodologies, ultimately leading to an integrated view of eukaryotic cell structure and function. Yeast remains an important testing ground for new tech-

B. Andrews, K. Willis

nological developments and conceptual advances in biomedical sciences. Presentations at the 2007 meeting made clear that yeast will remain at the forefront of model systems and will advance our understanding of eukaryotic cell biology for many years. In summary, this was an exciting and memorable meeting with attendees presenting some 225 scientific reports in 82 talks and 143 posters.

Funding for the meeting was provided by the CSHL Corporate Sponsor program.

PROGRAM

Chromosomes and Kinetochores
Chairperson: R. Rothstein, *Columbia University Medical Center, New York*

Cell Cycle and Division
Chairperson: M. Segal, *University of Cambridge, United Kingdom*

Trafficking
Chairperson: B. Wendland, *Johns Hopkins University, Baltimore, Maryland*

Cytoskeleton
Chairperson: J. Vogel, *McGill University, Montreal, Canada*

Polarity and Morphogenesis
Chairperson: R. Miller, *University of Rochester, New York*

Physiology and Genomics
Chairperson: D. Botstein, *Princeton University, New Jersey*

Organelle Biology
Chairperson: D. Amberg, *SUNY Upstate Medical University, Syracuse*

Signaling
Chairperson: J. Thorner, *University of California, Berkeley*

R. Strich, F. Luca

B. Vincent, R. Nash

E. Hong, J.M. Cherry, J. Park

Eukaroytic mRNA Processing

August 22–26 339 participants

ARRANGED BY **Douglas Black,** HHMI/University of California, Los Angeles
Timothy Nilsen, Case Western Reserve University
Joan Steitz, Yale University School of Medicine

This sixth meeting was arranged to present and discuss recent developments in mRNA metabolism. As in the past, regulated and alternative pre-mRNA splicing was a particular focus, as was the splicing mechanism. Additional areas included mRNA quality control and decay, mRNA trafficking, and 3′-end formation. Additional sessions covered computation analyses and genome-wide approaches and also the coupling of RNA processing to transcription and export.

In splicing regulation, there has been significant progress in deciphering the detailed regulatory mechanisms, as well as the biological roles of particular factors and splice variants. Individual groups have identified specific points in the spliceosome assembly pathway affected by particular factors. Another important trend that was touched on by a number of talks was new analytical methods by microarray and high-density sequencing that allow assessment of global changes in splicing. Finally, several talks examined the roles of splicing regulators in human disease and the development of splicing targeted therapeutics.

In the splicing mechanisms, several talks illuminated new proofreading mechanisms that function at the 5′ and 3′ splice sites. Other results indicated that different introns show differential dependence on constitutive splicing factors. Several talks examined the conformational changes occurring during the catalytic cycle of the spliceosome and the role of the RNA components of the spliceosome in catalysis. Important progress was also presented in structural studies of the different spliceosomal complexes. Several presentations on 3′-end formation focused on links between polyadenylation and transcription and quality control. Progress was described in characterizing the structure of the cleavage and polyadenylation complex and its individual components. Other talks examined the regulation of these processes by specific factors.

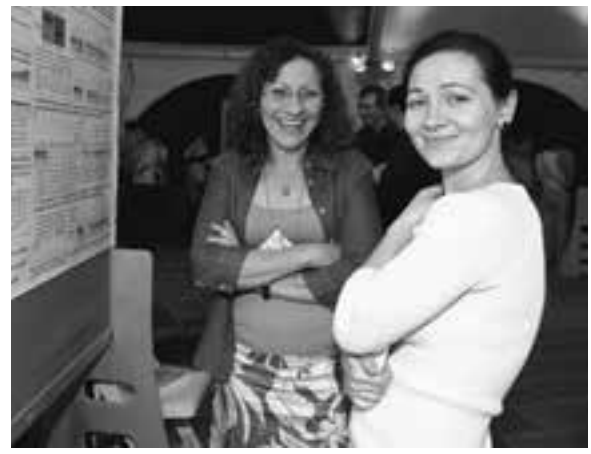
J. Steitz, T. Nilsen, D. Black

Talks in the RNA trafficking session examined factors involved in nuclear export of mRNAs. Other presentations described the localization of mRNAs in the cytoplasm and their stabilization and translational regulation. Another focus was the modification of RNA-bind-

C. Ortuno, J. Valdes, E. Garbarino-Pico

ing proteins and their cross-regulation of each other. In terms of surveillance and decay, significant progress was presented in understanding the role of the exon junction complex and its effects on translation and mRNA decay. Systems of inducing decay in specific sets of mRNAs or other RNAs were discussed. The control of deadenylation and hyperadenylation was another focus. Finally, several studies examined the coupling of transcription to RNA decay pathways.

This meeting was funded in part by the National Cancer Institute and the National Institute of Child Health and Human Development branches of the National Institutes of Health, and by the National Science Foundation. The Laboratory would in addition like to thank the RNA Society for its support of this meeting.



H. Loza-Tavera, V. Portnoy

PROGRAM

RNA Transport and Localization
Chairperson: K. Weis, *University of California, Berkeley*

Regulation of Alternative RNA Processing Patterns I
Chairperson: K. Lynch, *University of Texas Southwestern Medical Center, Dallas*

Global and Genome-wide Approaches to mRNA Processing Analysis
Chairperson: C. Burge, *Massachusetts Institute of Technology, Cambridge*

Splicing Mechanisms
Chairperson: C. Query, *Albert Einstein College of Medicine, Bronx, New York*

RNA Decay and RNA Surveillance
Chairperson: J. Lykke-Andersen, *University of Colorado, Boulder*

Special Lecture: Women In Science
J. Steitz, *Yale University School of Medicine*

Coupling of RNA Processing to Transcription
Chairperson: A. Kornblihtt, *University of Buenos Aires, Argentina*

Regulation of Alternative RNA Processing Patterns II
Chairperson: J. Caceres, *MRC Human Genetics Unit, Edinburgh, United Kingdom*

3′-end Maturation
Chairperson: C. Moore, *Tufts University School of Medicine, Boston, Massachusetts*

N.-C. Lau, R. Rounbehler

J. Potashkin, A. Krainer

Mechanisms of Eukaryotic Transcription

August 29–September 2 388 participants

ARRANGED BY **Barbara Graves,** Huntsman Cancer Institute, University of Utah
Steven Hahn, Fred Hutchinson Cancer Research Center
Jerry Workman, Stowers Institute for Medical Research

Regulation of gene transcription has a central role in the growth and development of eukaryotic organisms. Transcriptional responses occur as a consequence of cell signaling, environmental stresses, and developmental cues. Thus, the field of transcription encompasses a broad range of study from structural biology to developmental biology. This meeting appropriately covered all aspects of eukaryotic transcription and brought together a diverse group of scientists. This tenth meeting on this topic consisted of eight plenary sessions and two poster sessions.

S. Hahn, J. Tyler

The meeting began with a new session entitled "Genomics" that covered the wide range of mechanistic questions now being addressed at a genome-wide level. Next, a session focused on the mechanisms surrounding "Initiation and activation." A "Regulatory mechanism" session included nonconventional regulation of gene expression through microRNAs, variant histones, and nonpolymerase II examples. A session on "Elongation and termination" dramatically showed that regulation occurs beyond the transcription initiation step. Large multiprotein complexes that function in activation and chromatin remodeling were covered in two sessions. A "Signaling" session discussed the connections between DNA binding transcription factors and signal transduction pathways. A final session presented research using whole organisms to understand the use of a transcriptional mechanism for biological mechanisms in development and disease. The role of the plethora of histone marks was sprinkled throughout the sessions. The meeting was well-received by the participants and will occur again in 2009.

B. Graves

The meeting was funded in part by grants from the National Institutes of Health and the National Science Foundation.

PROGRAM

Genomics
Chairperson: J. Lis, *Cornell University, Ithaca, New York*

Initiation–Activation
Chairperson: C. Wolberger, *HHMI/Johns Hopkins University School of Medicine, Baltimore, Maryland*

Regulatory Mechanisms
Chairperson: H. Stunnenberg, *NCMLS, Radboud University, Nijmegen, The Netherlands*

Elongation–Termination
Chairperson: J. Tyler, *University of Colorado Health Science Center, Aurora*

Regulatory Complexes
Chairperson: M. Carey, *University of California School of Medicine, Los Angeles*

Chromatin-histone Modification
Chairperson: R. Kingston, *Massachusetts General Hospital, Harvard Medical School, Boston*

Signaling
Chairperson: M. Levine, *University of California, Berkeley*

Developmental Mechanisms
Chairperson: L. Tora, *IGMBC, Illkirch, France*

J. Workman, K. Zaret

K. Yamamoto, S. Block

CSHL/WELLCOME TRUST CONFERENCES

These conferences were held at the Hinxton Hall Conference Centre, located several miles south of Cambridge in the United Kingdom, which forms part of the Wellcome Trust Genome Campus, together with the Sanger Institute, and the European Bioinformatics Institute. The conferences are managed jointly by Cold Spring Harbor Laboratory and the Wellcome Trust and follow the Cold Spring Harbor style in that the majority of talks are chosen from openly submitted abstracts. The topics of the joint conference series emphasize genomics and bioinformatics, or topics of particular interest to science in the United Kingdom or Europe.

Interactome Networks

August 29–September 3 92 participants

ARRANGED BY **Ewan Birney,** European Bioinformatics Institute
Anne-Claude Gavin, European Molecular Biology Laboratory
Marc Vidal, Dana-Farber Cancer Institute/Harvard Medical School

This third conference was held at the Wellcome Trust Genome Campus in Hinxton, United Kingdom and attracted an audience of senior and junior investigators, postdoctoral, and (post) graduate researchers in a range of disciplines who sought to share existing research and experience. The conference addressed topics including ORFeome and other clone resources, Y2H and other binary assay maps; pull-down mass spectrometry approaches; orthogonal binary assays; assembly/annotation: integration with phenotypic, transcriptome and localization clustering data; domain-domain networks; and interaction-defective genetics. Keynote Speakers were Wolfgang Baumeister and Ed Harlow.

PROGRAM

Cocomplex Mapping I
Chairperson: M. Vidal, *Center for Cancer Systems Biology, Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts*

Keynote Address: Mapping Molecular Landscapes Inside Cells by Cryoelectron Tomography
W. Baumeister, *Max-Planck-Institute for Biochemistry*

Binary Mapping
Chairperson: A.-C. Gavin, *European Molecular Biology Laboratory, Heidelberg, Germany*

Cocomplex Mapping II
Chairperson: C. Sanderson, *University of Liverpool, United Kingdom*

Combining Networks
Chairperson: T. Davis, *University of Washington, Seattle*

Network Components/Interactome Modeling I
Chairperson: F. Roth, *Harvard Medical School, Boston, Massachusetts*

Interactome Modeling II
Chairperson: S. Wodak, *Hospital for Sick Children, Toronto, Canada*

Interactome Modeling III
Chairperson: S. Wodak, *Hospital for Sick Children, Toronto, Canada*

Keynote Speaker
E. Harlow, *Harvard Medical School*

Aerial view over the Francis Crick Auditorium and the Genome Campus. Sanger Institute visible in background.

Integrative Approaches to Brain Complexity

September 27–30 65 participants

ARRANGED BY **Seth Grant,** Wellcome Trust Sanger Institute
Nathaniel Heintz, HHMI/The Rockefeller University
Jeffrey Noebels, Baylor College of Medicine

This fourth conference addressed approaches ranging from sets of genes, from synapses to networked brain functions. Emphasis was placed on large-scale approaches that generate accessible molecular and biological databases. The conference was held at the Hinxton Hall Conference Centre, located several miles south of Cambridge in the United Kingdom, which forms part of the Wellcome Trust Genome Campus, together with the Sanger Centre, the European Bioinformatics Institute, and the HGMP-MRC Resource Center.

PROGRAM

Welcome and Outline of Meeting
S. Grant, *Wellcome Trust Sanger Institute*
N. Heintz, *HHMI/The Rockefeller University*
J. Noebels, *Baylor College of Medicine*

Mapping Visual Pathways I
Chairperson: N. Logothetis, *Max-Planck-Institute, Tübingen, Germany*

Mapping Visual Pathways II
Chairperson: T. Sejnowski, *Salk Institute for Biological Studies, La Jolla, California*

Molecular Mechanisms of Disease
Chairperson: J. Noebels, *Baylor College of Medicine, Houston, Texas*

Mapping Other Perceptual Pathways
Chairperson: G. Laurent, *California Institute of Technology, Pasadena*

Network Imaging
Chairperson: J. Rossier, *ESPCI-CNRS, Paris, France*

Molecular Networks
Chairperson: S. Grant, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Developmental Disorders
Chairperson: N. Heintz, *HHMI/The Rockefeller University, New York*

Network Synchrony and Ensembles
Chairperson: S. Grillner, *Karolinska Institutet, Stockholm, Sweden*

Wellcome Trust Conference Center: Main conference center and Hinxton Hall

Functional Genomics and Systems Biology

October 10–14 107 participants

ARRANGED BY **Alvis Brazma,** European Bioinformatics Institute
Thomas Freeman, University of Edinburgh
Katheleen Gardiner, Eleanor Roosevelt Institute

This first conference included oral presentations on mapping variations in humans and yeast; profiling of epigenetic marks and alternative mechanisms of transcription initiation in the early vertebrate embryo; and analysis of cell-based RNAi screens, to name a few. The conference followed a traditional format similar to traditional Cold Spring Harbor meetings, in that the majority of oral presentations were drawn from openly submitted abstracts.

PROGRAM

Session 1
Chairperson: A. Brazma, *European Bioinformatics Institute, Hinxton, United Kingdom*

Session 2
Chairperson: N. Luscombe, European *Bioinformatics Institute, Hinxton, United Kingdom*

Session 3
Chairperson: N. Luscombe, *European Bioinformatics Institute, Hinxton, United Kingdom*

Session 4
Chairperson: K. Gardiner, *University of Colorado, Denver*

Session 5
Chairperson: K. Gardiner, *University of Colorado, Denver*

Session 6
Chairperson: S. Teichmann, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Session 7
Chairperson: S. Teichmann, *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

Session 8
Chairperson: L. Serrano, *Centre for Genomic Regulation, Barcelona, Spain*

Session 9
Chairperson: L. Serrano, *Centre for Genomic Regulation, Barcelona, Spain*

Session 10
Chairperson: T. Freeman, *University of Edinburgh, United Kingdom*

Poster Sessions

Pharmacogenomics

October 17–20 140 particpants

ARRANGED BY **Alison Coffey,** Wellcome Trust Sanger Institute
Michel Eichelbaum, Institute of Clinical Pharmacology
Steve Leeder, Children's Mercy Hospital
Munir Pirmohamed, University of Liverpool
Dick Weinshilboum, Mayo Medical School
Roland Wolf, University of Dundee

This fifth conference focused on the opportunities presented by the growing contribution of emerging genomic information and technologies to interdisciplinary approaches in the study of variable responses of humans to drugs and toxic agents, and how research may benefit the individual. Topics covered an in-depth focus on diverse areas including the biochemistry and physiology of drug action, uptake, and metabolism, and how this affects genetics; the opportunities for discovery and design of new therapeutic agents; personalizing medicine; understanding and managing adverse drug reactions; the impact of academic and commercial initiatives; and ethical, legal, regulatory, and social consequences of genetics applied to medicines.

PROGRAM

Translation into Clinical Practice: Warfarin as a Paradigm
Chairpersons: R. Weinshilboum, *Mayo Clinic College of Medicine, Rochester, Minnesota;* A. Coffey, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Cancer Pharmacogenomics
Chairpersons: C.R. Wolf, *University of Dundee, United Kingdom;* A. Futreal, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Human Genome Variation
Chairperson: M. Hurles, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Pharmacogenetics in Neuropsychiatric Disease
Chairpersons: M. Eichelbaum, *Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany;* D. Goldstein, *Duke University, Durham, North Carolina*

Epidemiological Approaches in Pharmacogenomics
Chairpersons: A. Morris, *Ninewells Hospital & Medical School, Dundee, United Kingdom;* P. McKeigue, *University College Dublin, Ireland*

Adverse Drug Reactions
Chairpersons: M. Pirmohamed, *University of Liverpool, United Kingdom;* J.S. Leeder, *Children's Mercy Hospital, Kansas City, Missouri*

Conference Centre and Hall

Eukaryotic DNA Replication

September 5–9 331 participants

ARRANGED BY **Stephen Bell,** HHMI/Massachusetts Institute of Technology
Joachim Li, University of California, San Francisco

This was the ninth biannual meeting on eukaryotic DNA replication held at Cold Spring Harbor. Important progress has been made in our understanding of eukaryotic DNA replication, and this meeting is crucial in bringing together an international array of researchers investigating all aspects of eukaryotic DNA replication. The most recent advances in the field were presented together with new approaches for analyzing DNA replication, making this meeting the most important in the field. Investigators participated in the ten scientific sessions and platform and poster presentations, marked by spirited and enthusiastic exchanges of new results. In the scientific sessions, many audience members participated in the question and answer sessions, and the poster sessions were well-attended.

S. Bell, J. Li

Sessions at the meeting included assembly of replication initiation machines; replication timing and origin activation; genomic analysis of DNA replication; damage responses and checkpoint controls; replication and the cell cycle; replication fork proteins; control of replication during development; and origin selection. The meeting illustrated the rapid advances in our understanding of the function of replication proteins in initiation and elongation of DNA replication, the surveillance mechanisms monitoring the accuracy of replication, a global genomic view of replication, and the coordination between DNA replication and development.

Essential funding for the meeting was provided by the National Cancer Institute, a branch of the National Institutes of Health, and the National Science Foundation.

S. Chattopadhyay, D. Koepp

PROGRAM

From Structure to Function
Chairperson: L. Joshua-Tor, *Cold Spring Harbor Laboratory*

Origin Selection
Chairperson: B. Stillman, *Cold Spring Harbor Laboratory*

Replisome Assembly
Chairperson: K. Labib, *Cancer Research UK, Paterson Institute for Cancer Research, Manchester*

Cell Cycle Regulation and Connections
Chairperson: J. Blow, *University of Dundee, United Kingdom*

Special Session: NIH Review and Funding of Replication Grants
Chairperson: C. Dearolf, *NIGMS, National Institutes of Health, Bethesda, Maryland*

Damage and Replication Stress Responses
Chairperson: O. Aparicio, *University of Southern California, Los Angeles*

Chromatin Interactions and Developmental Control
Chairperson: G. Almouzni, *Institut Curie, Paris, France*

Origin Identification and Replication Timing
Chairperson: A. Donaldson, *University of Aberdeen, United Kingdom*

Fork Function During Replication and Repair
Chairperson: M. Foiani, *IFOM-FIRC Institute for Molecular Oncology, Milan, Italy*

W. Feng, J. Sidorova, I. Lucas

A.-K. Bielinsky, B. Calvi, C. Nieduszynski

P. Wong

M. Wattambe-Oda, Y. Li

Microbial Pathogenesis and Host Response

September 15–19 274 participants

ARRANGED BY **Brendan Cormack,** Johns Hopkins University School of Medicine
Theresa Koehler, University of Texas–Houston Medical School
James Slauch, University of Illinois, Urbana-Champaign

Despite advances in modern healthcare, infectious diseases continue to be major causes of human morbidity and mortality. The evolution of microbial pathogens with humans has resulted in complex interactions that impact the struggle between the infectious invader and the susceptible host. Increased understanding of these interactions with the goal of developing new therapeutics and preventive strategies requires collaborative and interdisciplinary scientific approaches. This meeting brought together a diverse group of international scientists who approach the study of bacterial and fungal pathogens from a broad range of perspectives. Investigators from the disciplines of molecular microbiology, eukaryotic cell biology, immunology, and genomics, and representing academia, industry, and the public health sector, shared recent findings concerning microbial and host aspects of infectious diseases.

N. Salama, B. Cormack

The meeting focused on bacterial and fungal pathogens and the host response and defense to these invading microbes. Oral sessions were topic-based and included studies of multiple and diverse organisms. Areas covered included effector delivery and function; regulation of virulence; microbial communities; immune response to pathogens; genomes and evolution of virulence; cell–cell communication; and microbial trafficking in cells and tissues. Speakers for each session were a mixture of established leaders in the field and young investigators. Several speakers, including some postdoctoral fellows and graduate students, were chosen from submitted abstracts. Dr. John Mekalanos, Professor and Chair of the Department of Microbiology and Molecular Genetics at Harvard Medical School presented the Keynote Address. Dr. Mekalanos is an internationally recognized leader in microbial pathogenesis. His talk, describing a newly discovered mechanism of bacterial protein delivery to eukaryotic cells, was followed by lively discussion centering on the similarities and differences between other protein delivery systems of pathogens.

J. Slauch

J. Mekalanos, A. Lothigius

The informal atmosphere combined with the broad perspectives of the meeting participants resulted in a free flow of novel and refreshing ideas on pathogenesis, with the atmosphere of a small meeting. Active questioning and discussion followed all oral presentations, was evident throughout the posters sessions, and continued during a wine and cheese reception and other social gatherings. We strongly encouraged submission of abstracts by junior researchers in the field and many young investigators were in attendance. Some of these interactions have already produced fruitful scientific collaborations.

This meeting was partially supported by funds from the National Institute of Allergy and Infectious Diseases and Burroughs Wellcome.

PROGRAM

Effector Delivery and Function
Chairperson: J. Galán, *Yale University, New Haven, Connecticut*

Regulation of Virulence
Chairperson: P. Sundstrom, *Dartmouth Medical School, Hanover, New Hampshire*

Cell Surfaces
Chairperson: B. Murray, *University of Texas Medical School, Houston*

Microbial Communities and Communication
Chairperson: V. Sperandio, *University of Texas Southwestern Medical School, Dallas*

Genomes and Evolution of Virulence
Chairperson: J. Heitman, *Duke University Medical Center, Durham, North Carolina*

Immune Response to Pathogens
Chairperson: N. Gow, *University of Aberdeen, United Kingdom*

Keynote Speaker
J. Mekalanos, *Harvard Medical School, Boston, Massachusetts*

Microbial Trafficking in Cells and Tissues
Chairperson: D. Russell, *Cornell University, Ithaca, New York*

P. Bandyopadhyay, E. Lang

A. Castillo, L. Forney

F. Cerda. C. White-Ziegler

Cell Death

September 26–30 325 participants

ARRANGED BY **J. Marie Hardwick,** Johns Hopkins University
Jürg Tschopp, University of Lausanne, Switzerland
Jungying Yuan, Harvard Medical School

J. Tschopp

This seventh Cold Spring Harbor meeting opened with two Keynote Addresses delivered by Eileen White and Hermann Steller. Steller's laboratory pioneered the *Drosophila* model of cell death and delineated the pathways that frame the field and this keynote address, in which he extended the knowledge gained from this model organism to the mechanisms of human disease and potential therapeutic targets. Eileen White, who originally demonstrated that inhibitors of apoptosis directly contribute to tumorigenesis, addressed the intertwined forces of apoptosis and autophagy in cancer. In addition to two poster sessions, there were eight oral sessions entitled Biochemical mechanisms of cell death, Mitochondria structure and function, Signaling pathways, Caspases and other proteases, Alternative death pathways, Disease models and systems biology, Multiple functions of cell death regulators in the nervous system, and New strategies and directions. On the basis of the research presented, strategies could be developed to treat a range of human disorders, from cancer to neurodegenerative disease. In all, the meeting featured 229 presentations and was attended by 325 participants representing more than 20 countries.

PROGRAM

Keynote Speaker
J.M. Hardwick, *Johns Hopkins University, Baltimore, Maryland*

Role of Apoptosis and Autophagy in Cancer
Chairperson: E. White, *Rutgers University*

Regulation of Caspases by Ubiquitin Pathway Proteins
Chairperson: Hermann Steller, *The Rockefeller University*

Biochemical Mechanisms of Cell Death
Chairperson: S. Kornbluth, *Duke University Medical Center, Durham, North Carolina*

C. Distelhorst, Y. Lazebnik

Mitochondrial Structure and Function
Chairpersons: R. Youle, *NINDS, National Institutes of Health, Bethesda, Maryland;* J. Nunnari, *University of California, Davis*

Cancer
Chairpersons: S. Lowe, *Cold Spring Harbor Laboratory;* K. Vousden, *Beatson Institute for Cancer Research, Glasgow, United Kingdom*

Signaling Pathways
Chairpersons: T. Mak, *Ontario Cancer Institute, Toronto, Canada;* S. Nagata, *Osaka University Medical School, Japan*

Caspases and Other Protease Pathways
Chairpersons: D. Green, *St. Jude Children's Research Hospital, Memphis, Tennessee;* G. Evan, *UCSF Comprehensive Cancer Center, San Francisco, California*

Alternative Death Pathways
Chairpersons: C. Thompson, *University of Pennsylvania, Philadelphia;* B. Stockwell, *Columbia University, New York*

Disease Models and Systems Biology
Chairpersons: E. White, *Rutgers University, Piscataway, New Jersey;* S. Cory, *Walter & Eliza Hall Institute of Medical Research, Melbourne, Australia*

Multiple Functions of Cell Death Regulators in the Nervous System
Chairperson: K. White, *Harvard Medical School, Boston, Massachusetts;* E. Jonas, *Yale University, New Haven, Connecticut*

New Strategies and Directions
Chairpersons: M.C. Simon, *University of Pennsylvania, Philadelphia;* B. Hay, *California Institute of Technology, Pasadena*

E. Shroff, C. Snyder

T. Grodzicker, E. White

J. Bertout, A. Bagchi

H. Steller, S. Larish

Neurobiology of *Drosophila*

October 3–7 418 participants

ARRANGED BY **Alex Kolodkin,** HHMI/The Johns Hopkins Medical School
Amita Sehgal, HHMI/University of Pennsylvania

As in previous years, the goal of this meeting was to foster communication of ideas, techniques, and new discoveries within the field of *Drosophila* neurobiology. The meeting was structured with platform and poster presentations by a variety of researchers including graduate students, postdoctoral fellows, and junior and senior faculty. The topics for the platform sessions were chosen to reflect the areas of *Drosophila* neurobiology in which cutting-edge advances are being made: neuronal and glial cell fate, neural circuits and function, technology development, cell biology and pathology, behavior, sensory systems, synaptic transmission, and process formation. A small number of abstracts submitted in each of these areas were selected by the respective session chairs for platform presentations, and the rest were presented as posters. The research reported used a wide range of techniques, including genetic, molecular, cellular, biochemical, physiological, and behavioral approaches to basic questions of nervous system development and function.

E. Kurant, A. Kolodkin

Among the highlights of the meeting were the creative ways in which researchers are using *Drosophila* to understand the molecular and cellular underpinnings of many different physiological and pathological processes. Efforts to humanize the fly are clearly proving to be successful in a number of areas such as the study of various behaviors, neurodegenerative disorders, and even the blood-brain barrier. In addition, the meeting included reports of important advances in the development of the nervous system, the perception of external stimuli, in particular olfactory cues, by the fly, and the design of new technology. The Elkins plenary lecture, which was presented by Richard Daniels from the Washington University School of Medicine, described exciting new findings on the properties of a vesicular glutamate transporter and its role in synaptic transmission. The environment of the meeting allowed many opportunities for informal discussions among all participants. The high quality of the presentations, the development of novel techniques, and the exciting new directions of *Drosophila* research demonstrate the vitality of this area. Discussions at the meeting led to cross-fostering of ideas that was valuable to everyone in the field.

This conference is supported in part by funds provided by the National Institute of Neurological Diseases and Stroke.

Y. Fang, A. Sehgal

PROGRAM

Neuronal and Glial Cell Fate
Chairperson: U. Gaul, *The Rockefeller University, New York*

Cell Biology and Pathology
Chairperson: L. Restifo, *University of Arizona, Tucson*

Elkins Memorial Lecture: The Function and Expression of the Vesicular Glutamate Transporter
Chairperson: R. Daniels, *Washington University School of Medicine*

Neural Circuits and Function/Technology
Chairpersons: J. Simpson, *HHMI/Janelia Farm Research Campus, Ashburn, Virginia;* G. Miesenböck, *Yale University, New Haven, Connecticut*

Sensory Systems/Technology
Chairpersons: K. Scott, *University of California, Berkeley;* G. Miesenböck, *Yale University, New Haven, Connecticut*

Behavior
Chairperson: F.R. Jackson, *Tufts University School of Medicine, Boston, Massachusetts*

Synaptic Transmission/Technology
Chairpersons: C.-F. Wu, *University of Iowa, Iowa City;* G. Miesenböck, *Yale University, New Haven, Connecticut*

Process Formation and Guidance
Chairperson: K. Zinn, *California Institute of Technology, Pasadena*

Watching closed circuit TV on Grace patio

T. Voller, D. Pauls

L. Griffith, J. Sierralta

J. Dorsten

Clinical Cardiovascular Genomics

October 10–13 168 participants

ARRANGED BY **David Ginsburg,** HHMI/University of Michigan Medical School
Elizabeth Nabel, National Heart, Lung and Blood Institute
Eddy Rubin, Lawrence Berkeley National Laboratory

This first Cold Spring Harbor meeting was opened by Keynote Addresses delivered by Michael Brown, Nobel laureate of Southwestern University Medical Center, and Eric Lander, of the Broad Institute of MIT and Harvard. The two speakers epitomize the union of clinical genetics with genomics. Brown's laboratory has pioneered investigations into the molecular basis of hypercholesterolemia, culminating in the fundamental discoveries of the LDL receptor and of the molecular mechanisms that coordinate its expression with cholesterol levels. Eric Lander has been one of the key driving forces behind the Human Genome Project and has contributed a wide array of tools and ideas enabling the use of genomics in studies of common human diseases. The opening session was followed by six oral sessions on Clinical genomics to function, Genome-wide association studies, Rare mutations as cause of common CV diseases, Analytical approaches, Large-scale clinical studies and resources and clinical models. In all, the meeting featured 83 presentations and was attended by participants from more than 20 countries.

This meeting was funded in part by the Foundation for the National Institutes of Health and NHLBI Programs for Genomic Applications.

D. Ginsburg, P. Gruber

E. Rubin, L. Pennacchio

M. Brown, E. Nabel

PROGRAM

Keynote Address
M.S. Brown, *University of Texas Southwestern Medical Center*

Clinical Genomics to Function
Chairpersons: C. Seidman, *Harvard Medical School, Boston, Massachusetts;* H. Dietz, *Johns Hopkins University, Baltimore, Maryland*

Genome-wide Association Studies
Chairpersons: D. Altshuler, *Harvard Medical School, Boston, Massachusetts;* D. Cox, *Perlegen Sciences Inc., Mountain View, California*

Rare Mutations as Cause of Common CV Diseases
Chairpersons: R. Lifton, *Yale University School of Medicine, New Haven, Connecticut;* L. Pennacchio, *Lawrence Berkeley National Laboratory, California*

Analytical Approaches
Chairpersons: E. Boerwinkle, *University of Texas, Houston;* G. Abecasis, *University of Michigan, Ann Arbor*

PGA Workshops

Large-scale Clinical Studies and Resources
Chairpersons: K. Stefansson, *deCode Genetics, Reykjavic, Iceland;* C. O'Donnell, *NHLBI Framingham Heart Study*

Keynote Address
E. Lander, *Broad Institute of MIT and Harvard*

Clinical Models
Chairpersons: D. Ginsburg, *HHMI/University of Michigan, Ann Arbor;* E. Topol, *Scripps Research Institute, La Jolla, California*

D. Vaidya, L. Yanek, J. Herrera

D. Cox

S. Eifert, M. Medina, I. Miljkovic Gacic

Genome Informatics

November 1–5 206 participants

ARRANGED BY **Michele Clamp,** Broad Institute of MIT and Harvard
Tim Hubbard, Wellcome Trust Sanger Institute
Jason Swedlow, University of Dundee

This seventh CSHL/Wellcome Trust conference continues to highlight the latest developments in genome research and, once again, was a vital and exciting meeting. This year, the conference was held at Cold Spring Harbor Laboratory, which is located on the North Shore of Long Island 35 miles east of Manhattan in New York State and is a research and educational institution with both a wide spectrum of research programs and a broad educational mission. The conference followed a traditional format similar to traditional Cold Spring Harbor meetings, in that the majority of oral presentations were drawn from openly submitted abstracts.

The explosion of biological data requires a concomitant increase in the scale and sophistication of information technology. This ranges from the storage of data and their associated data models, to the design of effective algorithms to uncover nonobvious aspects of these data sets, to ontologies to concisely describe biological information, and finally to software systems to support curation, visualization, and exploration. The conference brought together some of the leading scientists in this growing field, and researchers from other large-scale information-handling disciplines were also invited to attend. Topics included Epigenomics; Comparative and evolutionary genomics; Ontologies and definitions; Pathogenic microbe genomics; Regulation, pathways, and networks; Images, atlases, and reconstruction; and Assembly, annotation, and resources. For the first time, we had a session on Microbial Pathogens, a field that most bioinformaticians are unfamiliar with and which generated much excitement and discussion. The Keynote Speaker was Michael Ashburner, Department of Genetics, University of Cambridge. In all, more than 30% of delegates came from outside North America, a highly unusual statistic for a U.S. meeting, and the meeting hosted 140 scientific presentations in talks and posters.

J. Reimand, J. Witkowski, H. Peterson

PROGRAM

Regulation, Pathways, and Networks
Chairpersons: M. Kellis, *Broad Institute of Harvard and MIT, Cambridge, Massachusetts;* Z. Weng, *Boston University, Massachusetts*

Pathogenic Microbe Genomics
Chairperson: J. Carlton, *New York University, New York;* J. Parkhill, *Wellcome Trust Sanger Institute, Hinxton, United Kingdom*

Assembly, Annotation, and Resources I
Chairpersons: E. Birney, *European Bioinformatics Institute, Hinxton, United Kingdom;* J. Kent, *University of California, Santa Cruz*

Epigenomics
Chairpersons: R. Martienssen, *Cold Spring Harbor Laboratory;* T. Mikkelson, *Broad Institute of MIT and Harvard, Cambridge, Massachusetts*

Keynote Address: Birds of a Feather
M. Ashburner, *University of Cambridge*

Assembly, Annotation, and Resources II
Chairpersons: E. Birney, *European Bioinformatics Institute, Hinxton, United Kingdom;* J. Kent, *University of California, Santa Cruz*

Images, Atlases, and Reconstruction
Chairpersons: R. Murphy, *Carnegie Mellon University, Pittsburgh, Pennsylvania;* R. Waterston, *University of Washington, Seattle*

Comparative and Evolutionary Genomics
Chairpersons: R. Hardison, *Pennsylvania State University, University Park;* E. Margulies, *NHGRI, National Institutes of Health, Bethesda, Maryland*

S. Prochnik, J. Stajich

E. Margulies, P. Good

In Vivo Barriers to Gene Delivery

November 26–29 113 participants

ARRANGED BY **Nori Kasahara,** UCLA Geffen School of Medicine
Stephen Russell, Mayo Clinic College of Medicine
James M. Wilson, University of Pennsylvania

It is now 18 years since the first FDA-approved human gene transfer study, but this extremely promising field of endeavor is still nowhere close to realizing its full therapeutic potential. There have been a few notable successes, such as the use of autologous nematopoietic stem cells transduced with the IL-2γ chain or adenosine deaminase for the treatment of severe combined immunodeficiency. However, the dream of genetically based therapies for the treatment of a multitude of other more common human ailments has not yet been achieved. Although clinical "home runs" have been few and far between, the scientific understanding and technological underpinning of gene therapy have been moving forward in leaps and bounds, and there is currently a great deal of optimism that we are entering an era in which we will see increasing numbers of clinically approved gene therapy products. On the basis of this optimistic view of the field, it was decided that now would be an excellent time to initiate a new biannual series of Cold Spring Harbor meetings, focusing on the critical issue of in vivo barriers to gene delivery. The meeting was held the week after Thanksgiving and attracted many eminent field leaders and a strong showing of more junior scientists (more than 50% of attendees were postdoctoral scientists or Ph.D. students).

J. Chulay, J. Wilson

The first session focused on noninvasive monitoring of gene delivery and expression, which is considered to be a critical consideration in human gene therapy studies to give accurate insights into the reasons why some patients respond and others do not. The second session on mislocalization, sequestration, and targeting provided an opportunity to consider new vector targeting strategies as well as a number of interventions that can prevent vectors from becoming sequestered in the liver or spleen after intravenous administration. A third session focused on vector neutralization by complement proteins, antiviral antibodies, and other blood factors that serve to decrease the efficacy of vectors introduced by the intravenous route. The use of cells as nonneutralizable "Trojan horses" that can transport vectors to distant target sites was a recurring theme in this and other sessions. The fourth session was on vector persistence at the target site and dealt with the problem of rapid extinction of gene expression that is frequently encountered in gene therapy studies. The fifth session on extravasation and tissue penetration provided an opportunity to explore strategies whereby gene transfer vehicles can be induced to exit from the microcirculation and migrate within the interstitial fluid space. The last session was devoted to the key issue of vector manufacturing which, until now, has provided a significant bottleneck when moving from small-scale mouse studies to large-scale human studies.

S. Russell, D. Stewart

Overall, the meeting was considered a great success by those who attended. This was a highly interactive group of scientists leading to stimulating discussions after each of the oral presentations and

very lively interactions at the poster sessions and, of course, in the bar after each of the evening sessions. Throughout the meeting, it was clear that the field of gene therapy has reached a very exciting stage in its development and we expect to see significant progress addressing the in vivo barriers to gene delivery by the time of the next meeting in this series in 2009.

Funding for the meeting was provided by the CSHL Corporate Sponsor program.

PROGRAM

Monitoring Gene Delivery and Expression
Chairpersons: M. Barry, *Mayo Clinic, Rochester, Minnesota;* P. Leopold, *Weill Cornell Medical College, New York*

Mislocalization, Sequestration, and Targeting
Chairpersons: L. Seymour, *Cancer Research UK, University of Birmingham;* S. Russell, *Mayo Clinic College of Medicine, Rochester, Minnesota*

Vector Neutralization
Chairpersons: J. Wilson, *University of Pennsylvania, Philadelphia;* J. Bell, *Ottawa Health Research Institute, Canada*

Persistence
Chairpersons: K. High, *Children's Hospital of Philadelphia, Pennsylvania;* N. Kasahara, *UCLA Geffen School of Medicine, Los Angeles, California*

Extravasation and Tissue Penetration
Chairpersons: K. Bankiewicz, *University of California, San Francisco;* J. Wolff, *University of Wisconson, Madison*

Special Lecture
R. Crystal, *Weill Medical College of Cornell University*

Manufacturing
Chairpersons: M. Federspiel, *Mayo Clinic, Rochester, Minnesota;* K. Cornetta, *Indiana University Cancer Center, Indianapolis*

S. Alves, D. Hui, S. Murphy, F. Mingozzi, E. Basner-Tschakarjan

T. Ota, T. Ong

B. Schultz, A. Arnett

K. Breous, A. Chekmasova

Molecular and Immunological Approaches to Vaccine Design

November 29–December 2 52 participants

ARRANGED BY

Peter Beverley, Jenner Institute for Vaccine Research, United Kingdom
Kathrin Jansen, Wyeth Pharmaceuticals
Susan Swain, Trudeau Institute

This fifth winter conference set out to examine whether the revolution in understanding molecular and cell biology and new technologies that has taken place in recent years has begun to have a real impact on how new vaccines are designed. Sessions ranged from an intensive scrutiny of the function of Th17 CD4 T cells, through those on the generation of protective immune memory and effector mechanisms against infectious agents, to adjuvants, application of new technologies to difficult diseases, and the problems of immunizing the young and the old. At the level of the whole immunized animal, there remain gaps in understanding factors that determine how different types of protective immunities are generated and maintained in response to different organisms and in different tissue sites. Even in much-studied influenza virus models, the mechanisms underlying heterosubtypic (cross-protective) cellular immunity remain obscure, although it is clear that cellular response can provide a clinically useful level of protection in the absence of specific neutralizing antibody. Nevertheless, progress in understanding cellular immune responses is impressive, and the development of assays for detecting many cytokines simultaneously at the single-cell level is beginning to allow a much more refined analysis of the heterogeneity of responses and has focused attention on the importance of the quality as well as the magnitude of immune responses. An important conclusion is that every pathogen induces immunological memory with a unique functional profile. It is also clear that the recently appreciated cytokine, IL-17, has a role in memory T-cell responses to both bacterial and viral pathogens.

P. Beverley, L. Dorrell

L. Schultz, K. Jansen

The meeting heard how new technologies are showing encouraging results in difficult diseases. Prime boost immunization regimes using combinations of viral vectors are generating impressive responses to malaria antigens, suggesting that useful levels of protection may be achieved when different stages of the life cycle are targeted simultaneously. Identification of relatively conserved molecules of neisseria and staphylococci may lead to the development of new-generation vaccines for these organisms. In cancer, responses to tumor antigens can be achieved by immunization with internally adjuvanted DNA plasmids, but clinical trials of a therapeutic vaccine for HIV show how difficult it is to achieve success with therapeutic vaccines. Although the HIV vaccine induced strong responses, many of the responding cells had an exhausted phenotype and there was little effect on viral load.

The very young and the old present particular difficulties for vaccinologists. In the old, the immune system becomes increasing oligoclonal with a loss of diversity of the repertoire of naïve lymphocytes. Very large responses to a few pathogens, particularly cytomegalovirus, are generated. Exactly how this impacts on responses to other viruses in vivo remains to be determined. Nevertheless, in vitro assays of cellular immunity appear to provide useful prognostic information on susceptibility to influenza virus, again emphasizing its role in protection against a virus for which neutralizing antibody is considered to be of paramount importance. In the young, persistence of antibody responses is poor. The underlying cellular mecha-

S. Swain, B. Rocha

nism appears to be a failure of the bone marrow stroma to support survival of plasma cells responsible for long-term antibody production and the molecular defect a failure of young stromal cells to produce the TNF family member APRIL.

Although it is still difficult to understand the mechanisms of protection in many diseases, progress in applying an understanding of pattern-recognition receptors to develop effective adjuvants is an excellent example of the translation of fundamental understanding into practical applications. Appreciation of the need for better understanding of fundamental immune mechanisms to improve the rational design of vaccines is a goal of these conferences. The enthusiastic participation of attendees in discussions, in and outside the formal conference sessions, suggests that this goal was achieved.

Funding for the meeting was provided by the CSHL Corporate Sponsor program.

PROGRAM

CD4 T-cell Subsets and Immunity
Chairperson: P. Beverley, *Edward Jenner Institute for Vaccine Research, Compton, United Kingdom*

Generating Protective Immune Memory
Chairperson: S. Swain, *Trudeau Institute, Saranac Lake, New York*

Effector Mechanisms Against Infectious Agents
Chairperson: S. Swain, *Trudeau Institute, Saranac Lake, New York*

Adjuvants for Improved Vaccines
Chairperson: K. Jansen, *Wyeth Vaccine Research, Pearl River, New Jersey*

Application of New Technologies to Difficult Diseases I
Chairperson: K. Jansen, *Wyeth Vaccine Research, Pearl River, New Jersey*

Application of New Technologies to Difficult Diseases II
Chairperson: P. Beverley, *Edward Jenner Institute for Vaccine Research, Compton, United Kingdom*

Vaccines in the Young and the Old
Chairperson: P. Beverley, *Edward Jenner Institute for Vaccine Research, Compton, United Kingdom*

A. Shaw, P. Vink

A. Kajaste-Rudnitski, D. Ye

A. Sher

Rat Genomics and Models

December 6–9 131 participants

ARRANGED BY **Timothy Aitman,** Imperial College London
Norbert Hübner, Max-Delbrück-Center for Molecular Medicine
Anne Kwitek, University of Iowa
James Shull, University of Nebraska Medical Center

This successful fifth winter biotechnology conference has been held biannually since 1999 at Cold Spring Harbor Laboratory, with a complementary meeting outside of the United States (previously held in Europe, Japan, and Australia) in the alternating years. Drs. Hübner and Kwitek joined previous organizers, Drs. Shull and Aitman, in organizing this year's conference. The conference series is the first in the United States to focus exclusively on the strengths and uniqueness of the rat as a model organism for biological research, with an emphasis on leveraging genetic and genomic approaches. The primary goals of this meeting are to (1) promote interactions between biomedical researchers who utilize rat models in the study of physiology, pathophysiology, toxicology, immunology, neuroscience, and oncology, and (2) provide an interface between the research community and the various public and commercial resources that exist to support biomedical research in which rat models are utilized. For the first time for this meeting series, there was strong journal representation, with Editors attending from *Nature Genetics*, *Genome Research*, and *Physiological Genomics*.

F. Liska, N. Hubner

The meeting was organized into oral presentations by invited speakers/session chairs as well as speakers selected following evaluation of submitted abstracts, poster sessions, and workshops. There were also three outstanding Keynote Addresses from world leading physiologists, geneticists, and genome biologists: Allen Cowley, Jr., Richard Gibbs, and Ewan Birney. Two database workshops were given to provide tutorial to genome databases (RGD and Ensembl) and new Web tools (eQTL Explorer and MiMiR). Invited speakers by session topic included Drs. Jirout, Maatz, Michalkiewcz, Moreno, and Silva (Cardiovascular disease); Barkalifa, Behmoaras, Desarathy, Jagodic, Lazar, Mordes, Pravenec, and Redei (Metabolism and inflammation); Gould, Zschemisch, Adamovic, and Szpirer (Tumor biology); Johannason, Guryev, Lee, Liska, Morrissey, Ockinger, and Wallis (Comparative disease genomics); Kwitek,

A. Kwitek, E. Blankenhorn

T. Commers, J. Shull

Aitman, and Petretto (Systems biology, pharmacology, and toxicology); Pruitt and Twigger (Genome resources); Anegon, Liang, Mashimo, Stridh, and Teruel (Neuroscience); and Demers, Guertz, Kitada, and van Boxtel (Manipulating the rat genome). This meeting featured 103 presentations (60 oral and 43 posters) and attracted participants from around the world. This meeting had an exceptional international representation, with more than half of the participants traveling from outside the United States.

This meeting was funded in part by the National Heart, Lung, and Blood Institute, a branch of the National Institutes of Health.

PROGRAM

Cardiovascular Diseases
Chairpersons: A. Dominiczak, *University of Glasgow, United Kingdom;* N. Hübner, *Max-Delbrück-Center for Molecular Medicine, Berlin, Germany*

Keynote Address: Unraveling the Genomic Basis of Complex Functions and Diseases
A.W. Cowley, *Medical College of Wisconsin, Milwaukee*

Metabolism and Inflammation
Chairpersons: H. Markholst, *Hagedorn Research Institute, Gentofte, Denmark;* R. Holmdahl, *Lund University, Sweden*

Workshop I: Databases
Chairperson: S. Old, *NHLBI, National Institutes of Health, Bethesda, Maryland*

Tumor Biology
Chairpersons: J. Shull, *University of Nebraska Medical Center, Omaha;* T. Ushijima, *National Cancer Center Research Institute, Tokyo, Japan*

Keynote Address: What's Next for the Rat Genome?
R. Gibbs, *Baylor College of Medicine*

Comparative Disease Genomics
Chairpersons: P. Harris, *Mayo Clinic, Rochester, Minnesota;* T. Scheetz, *University of Iowa, Iowa City*

Workshop II: Databases and Tools
Chairperson: S. Old, *NHLBI, National Institutes of Health, Bethesda, Maryland*

Systems Biology, Pharmacology, and Toxicology
Chairpersons: H. Jacob, *Medical College of Wisconsin, Milwaukee;* C.R. Wolf, *University of Dundee, United Kingdom*

Keynote Address: The ENCODE Project—Understanding Mammalian Genomes
E. Birney, *European Bioinformatics Institute*

Genome Resources, Informatics
Chairpersons: T. Serikawa, *Kyoto University, Japan;* E. Cuppen, *Hubrecht Institute, Utrecht, The Netherlands*

Neuroscience
Chairpersons: J. Flint, *University of Oxford, United Kingdom;* T. Olsson, *Karolinska Institutet, Stockholm, Sweden*

Manipulating the Rat Genome
Chairpersons: Z. Izsvak, *Max-Delbrück-Center for Molecular Medicine, Berlin, Germany;* J. Mullins, *University of Edinburgh, United Kingdom*

M. Simakova, M. Jirout, M. Pravenec

J. Witkowski, R. Gibbs

M. Shimoyama, T. Serikawa

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 treat 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 11–24

INSTRUCTORS
K. Adelman, National Institutes of Health/NIEHS, Research Triangle Park, North Carolina
R. Burgess, University of Wisconsin, Madison
A. Courey, University of California, Los Angeles
S.-H. Lin, M.D. Anderson Cancer Center/University of Texas, Houston

ASSISTANTS
A. Clark, NIEHS, Research Triangle Park, North Carolina
M. Choueiri, M.D. Anderson Cancer Center, Houston, Texas
S. Duellman, University of Wisconsin, Madison
B. Glaser, University of Wisconsin, Madison
Y.-C. Lee, M.D. Anderson Cancer Center/University of Texas, Houston
S. Nechaev, NIEHS, Research Triangle Park, North Carolina
M. Nie, University of California, Los Angeles
N. Thompson, University of Wisconsin, Madison
W. Turki-Judeh, University of California, Los Angeles
C. Winkler, 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 includes laboratory work all day and a lecture with discussion session every evening. Each student became familiar with each of the major techniques in protein purification by actually performing four separate isolations including (1) a regulatory protein from muscle tissue, (2) a sequence-specific, DNA-binding protein, (3) a recombinant protein overexpressed in *E. coli*, and (4) a membrane-bound receptor.

A variety of bulk fractionation, electrophoretic, and chromatographic techniques discussed 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. In addition to the instructors, speakers in the 2007 course included CSHL investigators Leemor Joshua-Tor, Michael Myers, Bruce Stillman, and Nicholas Tonks.

PARTICIPANTS

Beebe, D., Ph.D., University of Wisconsin, Madison
Blewis, M., B.S., University of California, San Diego, La Jolla
Doctor, A., M.D., Washington University, St. Louis, Missouri
Franz, A., Ph.D., Harvard University, Cambridge, Massachusetts
Gupta, H., M.D., University of Alabama, Birmingham
Kao, H.-H., M.S., M.D. Anderson Cancer Center, Houston, Texas
Kuznetsov, V., B.S., Cancer Research UK London Research Institute, London, United Kingdom
Markelz, A., Ph.D., University at Buffalo, SUNY, Amherst, New York
Mosessian, S., B.S,, University of California, Los Angeles
Pastor, W., A.B., Harvard University, Boston, Massachusetts
Schmidt, R., B.A./M.S., University of California, Los Angeles
Simon, G., B.S., The Scripps Research Institute, La Jolla, California
Soh, H., Ph.D., University of California, Santa Barbara
Steinbrenner, J., Ph.D., University of Konstanz, Germany
Vershinin, M., Ph.D., University of California, Irvine
Wei, X., Ph.D., University of Pittsburgh, Pennsylvania

SEMINARS

Adelman, K., NIEHS/Research Triangle Park, North Carolina: RNA polymerase is poised for development across the genome.
Burgess, R., University of Wisconsin, Madison: Introduction to protein purification, immunoaffinity chromatography.
Burgess, R. and Glaser, B., University of Wisconsin, Madison: RNA polymerase-sigma factor interactions and use of LRET-based assays for drug discovery and biochemistry.
Courey, A., University of California, Los Angeles: Sumoylation: Links to epigenetic transcriptional control.
Joshua-Tor, L., Cold Spring Harbor Laboratory: Protein crystallography introduction and demonstration. DNA translocation in a relicative hexameric helicase.
Lin, S.-H., M.D. Anderson Cancer Center, Houston, Texas: Osteoblasts and prostate cancer metastasis.
Myers, M., Cold Spring Harbor Laboratory: Introduction to mass spectroscopy for analyzing biological samples.
Nechaev, S., NIEHS, Research Triangle Park, North Carolina: Analysis of promoter-proximal stalling.
Stillman, B., Cold Spring Harbor Laboratory: Role of ATP in replication origin recognition.
Tonks, N., Cold Spring Harbor Laboratory: Signal transduction from the protein tyrosine phosphatase perpective.

Cell and Developmental Biology of *Xenopus*

April 14–24

INSTRUCTORS **J. Heasman,** Children's Hospital Medical Center, Cincinnati, Ohio
C. Wylie, Children's Hospital Research Foundation, Cincinnati, Ohio

ASSISTANTS **A. Mir,** Children's Hospital Medical Center, Cincinnati, Ohio
M. Motta, Yale University School of Medicine, New Haven, Connecticut
Q. Tao, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio

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 covered included oocyte and embryo culture, lineage analysis, and experimental manipulation of embryos; gain- and loss-of-function analysis using mRNAs and antisense oligos, whole-mount in situ hybridization, immunocytochemistry, RT-PCR, and genomic/bioinformatic techniques; preparation of transgenic embryos; and use of *Xenopus tropicalis* for genetic analyses. This course was designed for those new to the *Xenopus* field, as well as for those wanting a refresher course in the emerging technologies. This course was open to investigators from all countries. Additional lecturers in the 2007 course included Daniel Buchholz, Ken Cho, Eddy de Robertis, Timothy Grammer, Raymond Keller, Mustafa Khokha, Paul Krieg, and Janet Weber.

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

Abbas, A., Ph.D., Era's Lucknow Medical College & Hospital, Lucknow, India
Baird, L., B.Sc., University of Bath, United Kingdom
Bayramov, A., Ph.D., Institute of Bioorganic Chemistry, Moscow, Russia
Charlesworth, A., Ph.D., University of Arkansas for Medical Sciences, Little Rock
Drews, C., M.Sc., University Clinic Essen, Essen, Germany
Gao, Q., M.D., Evanston Northwestern Healthcare Research Institute, Evanston, Illinois
Han, C.-L., Ph.D., Tri-Service General Hospital, Taipei, Taiwan
Harr, J., B.S., University of the Pacific, Stockton, California
Kong, S., Ph.D., Stowers Institute for Medical Research, Kansas City, Missouri
Marchal, L., M.S., Institut de Biology of Development de Marseille Luminy, Marseille, France
Mikryukov, A., M.S., Cancer Research Center of Laval University, Quebec City, Canada
Noble, A., Ph.D., University of Portsmouth, United Kingdom
Ochong, E., B.Sc., Liverpool School of Tropical Medicine, Liverpool, United Kingdom
Ren, X., M.S., Free University of Brussels, Gosselies, Belgium
Singh, A., Ph.D., University of Miami, Florida
Virnik, K., Ph.D., NCI/NIH, Bethesda, Maryland

SEMINARS

Buchholz, D., University of Cincinnati, Ohio: Metamorphosis.
Cho, K., University of California, Irvine: Cell adhesion and tissue assembly during *Xenopus* morphogenesis.
de Robertis, E., HHMI/University of California, Los Angeles: Early zygotic cell and tissue specification.
Grammer, T., University of Berkeley, California: *X. tropicalis* and genetic analysis of development 1.
Heasman, J., Children's Hospital Medical Center, Cincinnati, Ohio: Maternal control of *Xenopus* development.
Keller, R., University of Virginia, Charlottesville: Cell behavior during early morphogenesis.
Khokha, M., Yale University, New Haven, Connecticut: *X. tropicalis* and genetic analysis of development 2.
Krieg, P., University of Arizona College of Medicine, Tucson: Postgastrula organogenesis.
Weber, J., National Institutes of Health, Bethesda, Maryland: *Xenopus* genomics and bioinformatics.
Wylie, C., Children's Hospital Research Foundation, Cincinnati, Ohio: *Xenopus* as a model system.

Molecular Neurology and Neuropathology

June 6–12

INSTRUCTORS **M. Cookson,** National Institute on Aging/NIH, Bethesda, Maryland
J. Hardy, University College London, London, United Kingdom
H. Orr, University of Minnesota, Minneapolis

This intensive 1-week discussion course offered successful applicants a unique opportunity to learn the latest concepts and methodologies associated with the study of human neurological disorders such as Alzheimer's, Parkinson's, and epilepsy. Participants discussed in detail the strengths and weaknesses of the accumulated experimental evidence underlying our current understanding of these diseases. Fundamental questions—such as how and why particular neurons die in certain disorders—were discussed in the context of identifying the best experimental approaches to finding answers, whether through the use of transgenic and/or lesion-induced mouse models, functional brain and/or cellular imaging, gain-/loss-of-function molecular and viral approaches, cellular transplantation, or a combination of these approaches. The course examined why many of these disorders share apparently common features of protein aggregation, specific vulnerability of certain classes of neurons, and long incubation periods and discussed to what extent these features reflect common pathological mechanisms.

The course further explored how the underlying mechanisms in these disparate disorders are targeted for potential diagnostic and therapeutic gain. Extended seminars and discussion by a wide range of leading investigators further illuminated developments in this rapidly moving field. Participation in the course provided an essential conceptual and methodological framework for anyone intending to pursue rigorous research.

Speakers this year included Beverly Davidson (University of Iowa, Des Moines), Dennis Dickson (Mayo Clinic, Jacksonville, Florida), Monica Driscoll (Rutgers, The State University of New Jersey), Karen Duff (Columbia University, New York), Katrina Gwinn (NINDS, Bethesda, Maryland), Contantino Iadecola (Cornell University, New York), James McNamara (Duke University Medical Center), Jerome Siegel (Veterans Affair Greater L.A. Health Care System, California), Steven Warren (Emory University, Atlanta, Georgia), Mathias Heikenwälder (Institute for Neuropathology, Zurich, Switzerland), Rosa Rademakers (Mayo Clinic College of Medicine, Jacksonville, Florida), and Anthony Wynshaw-Borris (University of California, San Diego).

PARTICIPANTS

Adams, S., Ph.D., Mayo Clinic Jacksonville, Jacksonville, Florida
Aguirre, G., B.S., Cinvestav-IPN, Mexico City, Mexico
Ambegoakar, S., B.S., University of California, Los Angeles
Anvret, A., B.S., Karolinska Institute, Stockholm, Sweden
Chen, Y.-Y., B.S., Cold Spring Harbor Laboratory
De Paola, V., Ph.D., Cold Spring Harbor Laboratory
Gerris-Krol, H., B.S., Academical Medical Center, Amsterdam, The Netherlands
Gill, D., B.S., University of Prince Edward Island, Charlottetown, Canada
Micsenyi, M., B.S., Albert Einstein College of Medicine, New York
Morgenstern, N., B.S., Fundacion Instituto Leior, Buenos Aires, Argentina
Mukerji, S., B.S., Case Western Reserve University, Cleveland, Ohio
Pandey, U., Ph.D., University of Pennsylvania, Philadelphia
Reynolds, A., Ph.D., Princeton University, New Jersey
Scholz, S., Ph.D., National Institutes of Health, Bethesda, Maryland
Shelton, S., Ph.D., University of Cincinnati, Ohio
Shi, P., B.S., University of Kentucky, Lexington
Van Biervliet, J., Ph.D., KU Leuven, VIB, Herestraat, Belgium
Wider, C., Ph.D., Mayo Clinic College of Medicine, Jacksonville, Florida
Wood, R., Ph.D., University of Southern California, Los Angeles
Wu, J., B.S., University of California, Irvine
Yao, J., B.S., University of Southern California, Los Angeles
Zetterstrom, P., B.S., Umea University, Umea, Sweden

SEMINARS

Cookson, M., National Institute on Aging, Bethesda, Maryland: Parkinson's disease.
Davidson, B., University of Iowa, Des Moines: Gene manipulation therapy.
Dickson, D., Mayo Clinic Jacksonville, Florida: Neuropathological aspects of neurodegeneration.
Driscoll, M., Rutgers University, Piscataway, New Jersey: Invertebrate models.
Duff, K., Columbia University, New York: Models of Alzheimer's disease.
Gwinn, K., NINDS, Bethesda, Maryland: Clinical features of neurological disorders.
Hardy, J., University College London, United Kingdom: Genetics of neurodegenerative diseases.
Heikenwälder, M., Institute for Neuropathology, Zurich, Switzerland: The molecular biology of prion diseases.
Iadecola, C., Cornell University, Ithaca, New York: Stroke.
McNamara, J., Duke University, Durham, North Carolina: Neurotrophins and epileptogenesis.
Orr, H., University of Minnesota, Minneapolis: Triplet repeat disorders.
Rademakers, R., Mayo Clinic College of Medicine, Jacksonville, Florida: Tauopathies and other dementias.
Siegel, J., University of California, Los Angeles: Role of hypocretin (Orexin) in narcolepsy, Parkinson's, and normal behavior.
Warren, S., Emory University, Atlanta, Georgia: Fragile X.
Wynshaw-Borris, A., University of California, San Diego: Disorders of neuronal migration.

Advanced Bacterial Genetics

June 6–26

INSTRUCTORS **J. Kirby,** University of Iowa, Iowa City
S. Lovett, Brandeis University, Waltham, Massachusetts
A. Segall, San Diego State University, San Diego, California

ASSISTANTS **D. Ferullo,** Brandeis University, Natick, Massachusetts
C. Gunderson, San Diego State University, San Diego, California
M.C. Roper, University of Connecticut, Storrs
H. Vlamakis, Harvard Medical School, Boston, Massachusetts

The course presented logic and methods used in the genetic dissection of complex biological processes in eubacteria. Laboratory methods included classical mutagenesis using transposons, mutator strains, and chemical and physical mutagens; 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; and site-directed mutagenesis. Key components of the course were the use of sophisticated genetic methods in the analysis of model eubacteria, and the use of the wealth of new genomic sequence information to motivate these methods. Invited lecturers presented various genetic approaches to study eubacterial mechanisms of metabolism, development, and pathogenesis.

Speakers in the course included John Battista (Louisana State University and A&M College), David Botstein (Princeton University), Victor DiRita (University of Michigan Medical School), Susan Gottesman (National Cancer Institute), Alan Grossman (Massachusetts Institute of Technology), Kelly Hughes (University of Utah), Stanley Maloy (San Diego State University), and Thomas Silhavy (Princeton University).

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Aregger, R., M.S., University of Basel, Switzerland
Burger, L., M.S., University of Basel, Switzerland
Che, Y.-S., M.S., Osaka University, Japan
Dobrinski, K., B.S., University of South Florida, Tampa
Evans, T., B.A., University of Cambridge, United Kingdom
Galli, E., B.S., Newcastle University, United Kingdom
Grice, R., B.A., deCODE Biostructures, Bainbridge Island, Washington
Hartman, T., B.S., Albert Einstein College of Medicine, Bronx, New York
Iovine, N., Ph.D., New York University School of Medicine, New York
Iyengar, G., Ph.D., Columbia University, New York
Klebensberger, J., Diplom., University of Konstanz, Germany
Lovmar, M., Ph.D., Goteborg University, Sweden
Marks, M., Ph.D., Grinnell College, Grinnell, Iowa
Reimann, S., Ph.D., Loyola University Chicago, Maywood, Illinois
Shen, X., M.S., Harley McAdams and Mark Horowitz, Stanford, California
Yuan, J., Ph.D., Harvard University, Cambridge, Massachusetts

SEMINARS

Battista, J., Louisana State University and A&M College, Baton Rouge: Directed evolution of extreme radioresistance in *E. coli* strain MG1655.
Bostein, D., Princeton University, New Jersey: How the genome and the computer have changed biological science.
DiRita, V., University of Michigan Medical School, Ann Arbor: Genetic approaches to the study of two bacterial pathogens: *Vibrio cholerae* and *Campylobacter jejuni*.
Gottesman, S., National Cancer Institute, Bethesda, Maryland: Genetic dissection of small RNA roles in regulation.
Grossman, A., Massachusetts Institute of Technology, Cambridge: Control of horizontal gene transfer and recognition of self in *Bacillus subtilis*.
Hughes, K., University of Utah, Salt Lake City: Reducing the irreducible complexity of the bacterial flagellum.
Kirby, J., University of Iowa, Iowa City: Multicellularity and signal transduction in bacteria.
Lovett, S., Brandeis University, Waltham, Massachusetts: New mechanisms of replication fork repair: Template switch repair and the interplay of DNA damage with the SigmaS stress response.
Maloy, S., San Diego State University, California: Chromosome perversions in *Salmonella*.
Segall, A., San Diego State University, California: DNA repair, a novel target of antibiotics: A voyage from basic science to practical application.
Silhavy, T., Princeton University, New Jersey: Outer-membrane biogenesis in gram-negative bacteria.

Ion Channel Physiology

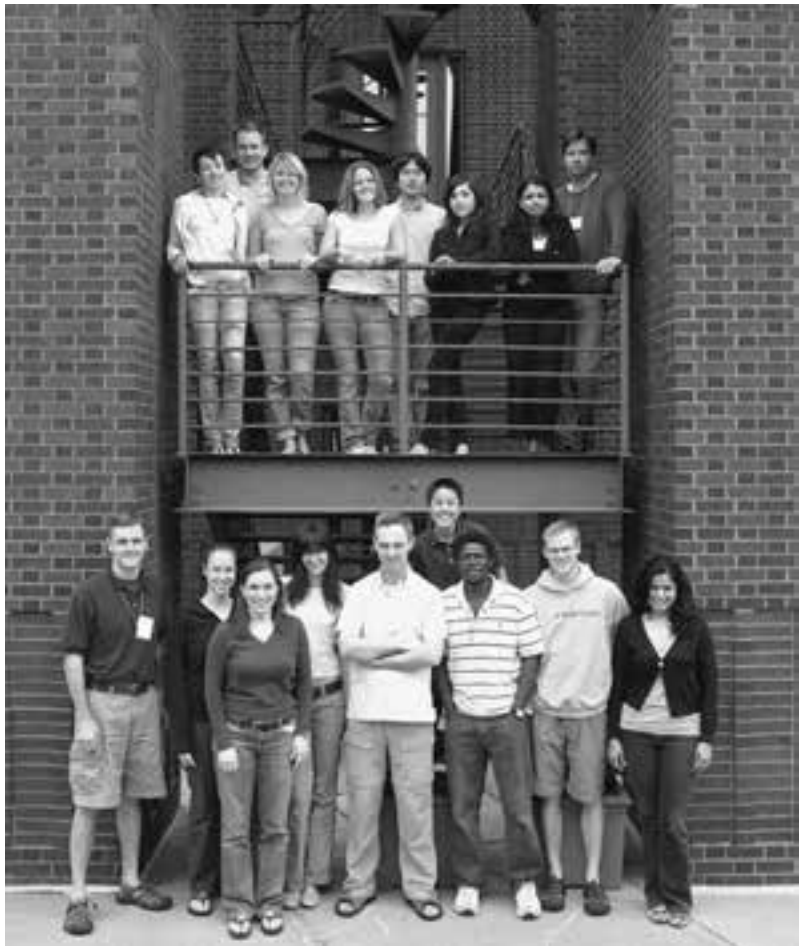
June 6–26

INSTRUCTORS **J. Diamond,** National Institutes of Health, Bethesda, Maryland
M. Farrant, University College London, United Kingdom
M. Hausser, University College London, United Kingdom

COINSTRUCTOR **K. Swartz,** National Institutes of Health, Bethesda, Maryland

ASSISTANTS **A. Alabi,**, NINDS, Bethesda, Maryland
R. Ali, University College London, United Kingdom
L. Kelly, University College London, United Kingdom
S. Moore, Northwestern University, Evanston, Illinois
A. Scimemi, National Institutes of Health, Bethesda, Maryland
B. Stell, Universite Paris 5, Paris, France

The primary goal of this course was to investigate through lectures and laboratory work the properties of ion channels that allow neurons to carry out their unique physiological functions in a variety of neural systems. Areas of particular interest included channels that (1) are activated by neurotransmitters at cen-



tral and peripheral synapses, (2) are activated by voltage changes in axons and dendrites, (3) respond to neuromodulators with changes in functional properties, and (4) are developmentally required and regulated. The research interests of guest lecturers 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 oocytes and in brain slice preparations. Different recording configurations were used to examine macroscopic or single-channel activity. The advantages and disadvantages of different methods, preparation, and recording techniques were considered in relation to the specific scientific questions being asked. Admissions priority was given to students and postdocs with a demonstrated interest, specific plans, and a supportive environment to apply these techniques to a defined problem.

Guest speakers included Clay Armstrong (University of Pennsylvania), John Issac (Developmental Synaptic/NINDS), Jeffry Isaacson (University of California, San Diego), Amy Lee (Emory University), Jeffrey Magee (LSUHSC), Steven Siegelbaum (Columbia University), Ivan Soltesz (University of California, Irvine), and Neson Spruston (Northwestern University).

This course was supported by the Howard Hughes Medical Institute.

PARTICIPANTS

Bhakar, A., Ph.D., Massachusetts Institute of Technology, Cambridge
Clemens, A., B.S., The University of Texas, Austin
Demir, E., Ph.D., Cold Spring Harbor Laboratory
Gertler, T., B.A., Northwestern University, Chicago, Illinois
Graham, D., B.S., Brown University, Providence, Rhode Island
Hiramoto, M., Ph.D., Cold Spring Harbor Laboratory
Hires, A., B.S., University of California, San Diego, La Jolla
Huang, W., B.A., University of California, San Francisco
Kozorovitskiy, Y., B.A., Princeton University, New Jersey
Martinez-Rubio, C., B.A., University of Puerto Rico, San Juan
Olveczky, B., Ph.D., Harvard University/MIT, Cambridge, Massachusetts
Parekh, R., B.A., Pennsylvania State University, University Park

SEMINARS

Armstrong, C., University of Pennsylvania, Philadelphia: The gating of ion channels.
Hausser, M., University College London, United Kingdom: Synaptic integration.
Isaac, J., Developmental Synaptic/NINDS, Bethesda, Maryland: Development of the layer-4 barrel cortex circuit.
Isaacson, J., University of California, San Diego, La Jolla: Microcircuits.
Lee, A., Emory University, Atlanta, Georgia: Macromolecular regulation of voltage-gated Ca^{+} channels.
Magee, J., Louisiana State University Health Sciences Center, New Orleans: Dendrites.
Siegelbaum, S., Columbia University, New York: Hyperpolarization-activated cation channels and their function in neural activity.
Soltesz, I., University of California, Irvine: Activity-dependent plasticity of endocannabinoid signaling.
Spruston, N., Northwestern University, Evanston, Illinois: Dendritic integration and synaptic plasticity.
Swartz, K., NINDS/NIH, Bethesda, Maryland: K^{+} channel structure, permeation mechanisms, and gating. Mechanisms of voltage sensing.

Molecular Embryology of the Mouse

June 6–26

INSTRUCTORS **D. Threadgill,** University of North Carolina, Chapel Hill
P. Trainor, Stowers Institute for Medical Research, Kansas City, Missouri

COINSTRUCTORS **V. Pachnis,** National Institutes for Medical Research, London, United Kingdom
L. Pevny, University of North Carolina, Chapel Hill

ASSISTANTS **S. Hutton,** University of North Carolina, Chapel Hill
A. Iulianella,, Stowers Institute for Medical Research, Kansas City, Missouri
D. Natarajan, University of Cambridge, London, United Kingdom
J. Rivera-Perez, University of Massachusetts Medical School, Worcester
L. Sandell, Stowers Institute for Medical Research, Kansas City, Missouri

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 an extensive hands-on introduction to mouse embryo analysis. Experimental techniques included in vitro culture and manipulation of preimplantation and postimplantation embryos, embryo transfer, assisted reproduction and cryopreservation, culture and genetic manipulation of embryonic stem cells, production of chimeras by embryo aggregation and by ES cell injection, and transgenesis by pronuclear microinjection and nuclear cloning. In addition, this year's practicals featured increased emphasis on phenotypic analysis of mutants, including techniques of histology, in situ hybridization, immunohistochemistry, skeletal preparation, vascular casting, organ culture, and tissue recombination.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Afelik, S., Ph.D., University of Colorado Health Sciences Center, Aurora
Aravin, A., Ph.D., Cold Spring Harbor Laboratory
Cai, L., B.S., University of North Carolina, Chapel Hill
Gaengel, K., Ph.D., Karolinska Institutet, Stockholm, Sweden
Garcia Gonzalo, F., Ph.D., The Salk Institute for Biological Studies, La Jolla, California
Gebeshuber, C., M.S., Institute of Molecular Biology, Vienna, Austria
Kostanova Pollakova, D., Ph.D., Institute of Molecular Biology, Vienna, Austria
Liu, J., B.A., Wesleyan University, Middletown, Connecticut
Mommersteeg, M., M.S., University of Amsterdam, The Netherlands
Ren, X., M.S., Free University of Brussels, Gosselies, Belgium
Ryan, N., B.S., Medical Research Council, London, United Kingdom
Saze, H., Ph.D., National Institutes of Genetics, Japan
Shi, J., Ph.D., Novartis Institute of Biomedical Research, Cambridge, Massachusetts
Wu, V., Ph.D., University of California, San Francisco

SEMINARS

Behringer, R., The University of Texas/M.D. Anderson Cancer Center, Houston: Transgenics and insertional mutants.
Hadjantonakis, K., Memorial Sloan-Kettering Cancer Center, New York: Mouse imaging.
Hochedlinger, K., Harvard Medical School and Harvard Stem Cell Institute, Boston, Massachusetts: Nuclear reprogramming.
Justice, M., Baylor College of Medicine, Houston, Texas: ENU mutagenesis screens and chromosome engineering.
Lovell-Badge, R., MRC National Institute for Medical Research: Sex determination.
Magnuson, T., University of North Carolina, Chapel Hill: Epigenetics.
Majesky, M., Baylor College of Medicine, Houston, Texas: Vasculogenesis.
McLaren, A., University of Cambridge, United Kingdom: Germ cells.
McMahon, A., Harvard University, Cambridge, Massachusetts: Kidney organogenesis.
Mombaerts, P., The Rockefeller University, New York: Olfactory system.
Nagy, A., Samuel Lunenfeld Research Institute, Toronto, Canada: Gene targeting.
Ornitz, D., Washington University, St. Louis, Missouri: Skeletal biology.
Pachnis, V., National Institutes for Medical Research, London, United Kingdom: Enteric neural crest, gut, intestine development.
Parada, L., University of Texas Southwestern Medical Center, Dallas: Cancer tumor.
Pevny, L., University of North Carolina, Chapel Hill: Neural stem cells neurogenesis.
Pfaff, S., The Salk Institute, La Jolla, California: Spinal cord neurogenesis.
Rosenthal, N., European Molecular Biology Laboratory, Monterortondo-Scala, Italy: Heart development.
Sherr, C., HHMI/St. Jude Children's Research Hospital, Memphis, Tennessee: Tumorigenesis, p53, Arf.
Solter, D., Max-Planck-Institut für Immunobiologie, Freiburg, Germany: Preimplanation development.
Soriano, P., Fred Hutchinson Cancer Research Center, Seattle, Washington: PDGF, Eph/Ephrin signaling.
Stewart, C., National Cancer Institute, Frederick, Maryland: ES cells.
Tajbakhah, S., Pasteur Institute, Paris, France: Myogenesis and muscle satellite stem cells.
Takahashi, J., HHMI/Northwestern University, Evanston, Illinois: Behavioural genetic screens and development.
Tam, P., Children's Medical Research Institute, Sydney, Australia: Introduction to mouse development. Gastrulation and formation of the body plan.
Threadgill, D., University of North Carolina, Chapel Hill: Quantitative traits.
Trainor, P., Stowers Institute for Medical Research, Kansas City, Missouri: Neural crest cells and crainofacial development.
Woychik, R., The Jackson Laboratory, Bar Harbor, Maine: Mouse genetics.

Integrated Data Analysis for High-throughput Biology

June 13–26

INSTRUCTORS **H. Bussemaker,** Columbia University, New York
V. Carey, Harvard University, Boston, Massachusetts
P. Mitra, Cold Spring Harbor Laboratory
M. Reimers, National Cancer Institute, Bethesda, Maryland

ASSISTANTS **G. Halasz,** Columbia University, New York
P. Gomez-Alcala, Columbia University, New York
D.W. Lucas, Columbia University, New York
J. Mar, Harvard University, Cambridge, Massachusetts
J. Zhang, Dana-Farber Cancer Institute, Walpole, Massachusetts

High-throughput biology, epitomized by the ubiquitous DNA microarray, is rapidly generating enormous observation sets. Biologists seeking to make sense of this growing body of data need to have a firm grasp of statistical methodology. This course was designed to build competence in quantitative methods for the analysis of high-throughput molecular biology data, from which meaningful inferences about biological processes can be drawn. It also included the following:

- Review of multivariate statistics.
- R minitutorial.
- Expression and other microarrays: experimental design, scanning and image analysis, quality control, normalization and probe-level analysis for spotted arrays or prefabricated chips, exploratory analysis, tests of significance and multiple testing, using R and the Bioconductor.
- Discrimination and classification of samples.
- Identifying general regulation themes (e.g., Gene Ontology categories) in gene lists by statistical means.
- Promoter analysis in yeast using ChIP and expression data.

- Identifying regulatory polymorphisms using SNP and expression data.
- Characterizing the effect of DNA amplifications and deletions on gene expression in cancer using CGH and expression data on the same samples.

Speakers in this year's course included Keith Baggerly (M.D. Anderson Cancer Center), Benilton Carvalho (The Johns Hopkins University), Vivian Cheung (University of Pennsylvania), Aedin Culhane (Dana Farber Cancer Institute/Harvard), Bruce Futcher (Stony Brook University), Jason Lieb (University of North Carolina, Chapel Hill), Dana Pe'er (Columbia University), Anirvan Sengupta (Rutgers University), Michael Snyder (Yale University), Richard Spielman (University of Pennsylvania), Danielle Thierry-Mieg (National Institutes of Health), Jean Thierry-Mieg (National Institutes of Health), and John Zhang (Dana-Farber Cancer Institute).

The first week of the course concentrated on analysis of specific types of microarray data (expression, Affymetrix, CGH, ChIP-chip, and SNP arrays) and proteomics. The second week explored biological problems involving the integration of several types of high-throughput data. Data sets were drawn from yeast, human polymorphisms, and cancer biology.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Chicas, A., Ph.D., Cold Spring Harbor Laboratory
Doherty, C., B.A., Michigan State University, E. Lansing
Ghavihelm, Y., M.S., CEA/Saclay, Gif sur Yvette, France
Margolis, R., Ph.D., NIDDK/NIH, Bethesda, Maryland
Meza-Zepeda, L., Ph.D., Rikshospitalet-Radiumhospitalet Medical Center, Oslo, Norway
Miller, J., B.S., University of California, Los Angeles
Mohanty, S., Ph.D., ETH Zurich, Switzerland
Ni, X., B.S., The University of Chicago, Illinois
Nickles, D., Dipl., German Cancer Research Institute, Heidelberg, Germany
Palta, P., B.Sc., University of Tartu, Estonia
Speers, C., B.S., Baylor College of Medicine, Houston, Texas
Tantisira, K., M.D., Brigham and Women's Hospital, Boston, Massachusetts
Wang, W., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Wichert, S., Dipl., Max-Planck-Institute for Experimental Medicine, Goettingen, Germany
Yang, B.-Z., Ph.D., Yale University, West Haven, Connecticut
Zheng, Y., Ph.D., Stanford University, Stanford, California
Zhou, Y., Ph.D., Fox Chase Cancer Center, Philadelphia, Pennsylvania

SEMINARS

Baggerly, K., M.D. Anderson Cancer Centre, Houston, Texas: Reverse-phase protein arrays.
Bussemaker, H., Columbia University, New York: Interfering condition-specific activity of TFs. In vivo versus in vitro target specificity. The importance of posttranscriptional regulation.
Cheung, V., University of Pennsylvania, Philadelphia: Genetics of gene expression.
Culhane, A., Dana Farber Cancer Institute/Harvard, Cambridge, Massachusetts: Exploratory analysis and ordination.
Futcher, B., Stony Brook University, New York: Analysis of cell cycle data in yeast.
Lieb, J., University of North Carolina, Chapel Hill: Microarray technology. ChIP arrays and histones.
Lucito, R., Cold Spring Harbor Laboratory: Methylation arrays.
Pe'er, D., Columbia University, New York: Module networks.
Reimers, M., Virginia Commonwealth University, Richmond: Microarray preprocessing—QA and normalization. Epigenomic data analysis.
Reimers, M., Virginia Commonwealth University, Richmond, and Carvalho, B., The Johns Hopkins University, Baltimore, Maryland: Oligonucleotide arrays.
Sengupta, A., Rutgers University, Piscataway, New Jersey, and Bussemaker, H., Columbia University, New York: Quantifying the sequence specificity of TFs.
Snyder, M., Yale University, New Haven, Connecticut: ChIP-chip and/or protein arrays.
Spielman, R., University of Pennsylvania, Philadelphia: Genetical genomics.
Thierry-Mieg, J. and Theirry-Mieg, D., National Institutes of Health, Bethesda, Maryland: MAQC. Alternate splicing (and tiling arrays?).
Threadgill, D., University of North Carolina, Chapel Hill: Genetical genomics via the collaborative cross.
Wigler, M., Cold Spring Harbor Laboratory: CGH in cancer.
Zhang, J., Dana-Farber Cancer Institute, Boston, Massachusetts: Methylation.

Workshop on Autism Spectrum Disorders

June 14–21

INSTRUCTORS **D. Geschwind,** University of California, Los Angeles
P. Levitt, Vanderbilt University, Nashville, Tennessee
S. Spence, National Institute of Mental Health, Bethesda, Maryland

Autism spectrum disorders (ASDs) are developmental disorders with complex phenotypes defined by a triad of symptoms that include disrupted social abilities, verbal and nonverbal communication skills, and restricted interests with repetitive behaviors. Co-occurring neurological and medical conditions often occur in these disorders. The underlying etiology remains a mystery, but ASDs are the most highly heritable of neuropsychiatric disorders. This workshop examined dimensions of ASD on various levels, including sessions on characteristics of the clinical syndrome; neuropathology, imaging, and cognitive neuroscience studies that implicate circuits and systems involved in ASD; the current state of findings from human genetics; concepts regarding the developmental neurobiological basis of ASD; and the use of experimental models and current etiological theories and hypotheses of ASD.

In addition to hearing about the most recent research in these areas, we explored and debated controversial topics and challenges to basic assumptions in the field. An exceptional faculty with diverse interests brought the most up-to-date results and theories to the students, making this workshop a valuable resource for young researchers starting out in this fast-moving and expansive field. Not only did it help them build the foundation for their future research, but it also introduced them to many potential collaborators working to understand ASD from different disciplines.

Speakers this year included B.J. Casey (Weill Medical College at Cornell University, New York), Richard Davidson (University of Wisconsin, Madison), Eric Fombonne (McGill University, Toronto, Canada), Takao Hensch (Harvard University, Cambridge, Massachusetts), Portia Iverson (Cure Autism Now, Los Angeles, California), Ami Klin (Yale University School of Medicine, New Haven, Connecticut), David Ledbetter (Emory University School of Medicine, Atlanta, Georgia), Carlos Pardo-Villamizar (Johns Hopkins University School of Medicine, Baltimore, Maryland), Richard Paylor (Baylor College of Medicine, Houston, Texas), Isaac Pessah (University of California, Davis), Isabelle Rapin (Albert Einstein School of Medicine, Bronx, New York), and Jeffrey Rogers (Southwest Foundation for Biomedical Research, San Antonio, Texas).

PARTICIPANTS

Addante, R., B.S., University of California, Davis
Aldinger, K., B.S., Univeristy of Chicago, Illinois
Alonso, A., Ph.D., New York State Institute for Basic Research, Staten Island
Bhatara, A., B.S., McGill University, Montreal, Canada
Bill, B., B.S., University of Minnesota, Burnsville
Bowers, K., B.S., Johns Hopkins University, Washington D.C.
Carpenter, K., B.S., University of North Carolina, Chapel Hill
Chukoskie, L., Ph.D., Salk Institute, La Jolla, California
Cubells, J., Ph.D., Emory University, Atlanta, Georgia
Dinstein, I., B.S., New York University, New York
Duo, J., Ph.D., Kanazawa University, Kanazawa, Japan
Gjevik, E., Ph.D., Ulleval University Hospital, Oslo, Norway
Herzing, L., B.S., Northwestern University, Evanston, Illinois
Hoffman, E., Ph.D., Mount Sinai School of Medicine, New York
Kumar, R., Ph.D., University of Chicago, Chicago, Illinois
Lenroot, R., Ph.D., National Institutes of Health, Bethesda, Maryland
Lieberman, D., Ph.D., Johns Hopkins University, Baltimore, Maryland
Maffie, J., B.S., New York University, New York
Pasca, S., B.S., I. Hatieganu University, Cluj-Napoca, Romania
Payne, C., B.S., Emory University, Atlanta, Georgia
Rehnstrom, K., B.S., National Public Health Institute, Helsinki, Finland
Smith, S., B.S., California Institute of Technology, Pasadena
Walker, J., B.S., University of Texas Southwestern Medical Center, Dallas

SEMINARS

Casey, B.J., Weill Medical College at Cornell University, New York: Introduction to imaging. Imaging from a neural systems perspective.
Fombonne, E., McGill University, Montreal, Canada: Autism epidemiology, part I and part II.
Geschwind, D., University of California, Los Angeles: Neuropathology and introduction to neural systems.
Klin, A., Yale University, New Haven, Connecticut: Clinical presentation, core features, measures. Autism spectrum disorders broader phenotypes, phenotypic variability, and outcomes.
Levitt, P., Vanderbilt University, Nashville, Tennessee: Introduction to developmental neurobiology.
Levitt, P., Vanderbilt University, Nashville, Tennessee: Neuroanatomical systems implicated in autism spectrum disorders.
Rapin, I., Albert Einstein School of Medicine, Bronx, New York: History of autism spectrum disorders.
Spence, S., National Institutes for Mental Health, Bethesda, Maryland: Clinical assessments. Medical models/ comorbidities. Treatment overview. Endophenotypes—Language delay, epilepsy, regression, OFC.

Workshop on Mechanisms of Alertness, Arousal, and Attention

June 22–29

INSTRUCTORS **B. Kieffer,** Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France
D. Pfaff, The Rockefeller University, New York

Brain mechanisms for arousal, alertness, and attention underlie and are necessary for all cognitive functions and all emotional expression. This course covered what is known about neuroanatomy, neurophysiology, and functional genomics and explored the medical consequences of their damage in human patients, e.g., vegetative states, attention deficit hyperactivity disorder, autism, age-related dementias, mood disorders, and sleep disorders. Public health problems related to vigilance, environmental toxins, and fatigue states were of interest. Relevant techniques were discussed, with an emphasis on molecular genetics on neurochemistry and on human and animal behavior. Closely related topics such as circadian rhythms and sleep/wake physiology were included.

Speakers this year included Don Pfaff (The Rockefeller University), Mark Bellgrove (University of Queensland, Australia), Robert Moyzis (University of California, Irvine), Turhan Canli (Stony Brook University), Gary Aston-Jones (University of South Carolina), Cliff Kentros (University of Oregon), Nicholas Schiff (Cornell Medical School), Steven Laureys (University of Liege, Belgium), Tor Wager (Columbia University), Brigitte Kieffer (University of Strasbourg, France), Joseph Takahashi (HHMI/Northwestern University), Rae Silver (Columbia University), Ronald Szymusiak (University of California, Los Angeles), Allan Pack (University of Pennsylvania Medical School), Justin Blau (New York University), and Steve Brown (University of Zurich, Switzerland).

PARTICIPANTS

Burgos-Ruiz, E., B.S., Georgetown University, Washington, D.C.
Clerkin, S., B.S., City University of New York, Yonkers
Davis, J., B.S., University of British Columbia, Vancouver, BC, Canada
Haley, D., Ph.D., University of Toronto, Canada
Itoi, K., Ph.D., Tohoku University, Sendai, Japan
Keating, G., Ph.D., Emory University School of Medicine, Atlanta, Georgia
Larkin, E., B.S., Case Western Reserve University, Cleveland, Ohio
McVay, J., B.S., University of North Carolina, Greensboro
Melloni, L., Ph.D., Brain Imaging Center, Frankfurt, Germany
Mooshagian, E., B.S., University of California, Los Angeles
Panos, J., B.S., Western Michigan University, Kalamazoo
Roos, A., B.S., University of Stellenbosch, Tygerberg, South Africa
Vosko, A., B.S., University of California, Los Angeles

SEMINARS

Aston-Jones, G., University of South Carolina, Charleston: Arousal and motivational systems involved in reward-seeking.
Bellgrove, M., University of Queensland, Australia: Molecular genetics of attention.
Blau, J., New York University, New York: Transcriptional and posttranscriptional mechanisms running the circadian clock.
Brown, S., University of Zurich, Switzerland: Clocks throughout the body: Interesting mechanisms and powerful tools.
Canli, T., Stony Brook University, New York: Genes related to affect.
Kentros, C., University of Oregon, Eugene: Attentional modulation of hippocampal representation of information.
Kieffer, B., Institut de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France: Genetic influences on opiodergic systems in the CNS and their importance for reward.
Laureys, S., University of Liege, Belgium: Imaging of CNS responses in patients with impaired levels of consciouness.
Moyzis, R., University of California, Irvine: Genes influencing attention deficit disorders.
Pack, A., University of Pennsylvania Medical School, Philadelphia: Genetic contributions to the control of sleep.
Pfaff, D., The Rockefeller University, New York: Introduction. Mechanisms underlying generalized CNS arousal.
Schiff, N., Weill Medical College of Cornell University, New York: Disorders of consciousness in neurological patients.
Silver, R., Columbia University, New York: Suprachiasmatic nerve cells governing fluctuations in arousal, alertness, and attention.
Szymusiak, R., University of California, Los Angeles: Preoptic/hypothalamic nerve cells influencing temperature, sleeping, and waking behavior.
Takashashi, J., Northwestern University, Evanston, Illinois: Transcriptional controls over circadian rhythms.
Wager, T., Columbia University, New York: Neural bases of placebo effects and pains.

Advanced Techniques in Molecular Neuroscience

June 29–July 15

INSTRUCTORS
J. Eberwine, University of Pennsylvania Medical School, Philadelphia
J. Kohler, Stanford University, California
C. Lai, Scripps Research Institute, La Jolla, California
R. Lansford, California Institute of Technology, Pasadena

ASSISTANTS
T. Bell, University of Pennsylvania School of Medicine, Philadelphia
C. Challis, Scripps Research Institute, La Jolla, California
D. Huss, California Institute of Technology, Pasadena
T. Peritz, University of Pennsylvania Medical Center, Philadelphia
G. Poynter, California Institute of Technology, Pasadena
M. Samson, Harvard Medical School/HHMI, Boston, Massachusetts
T. Young-Pearse, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts

This newly revised laboratory and lecture course was designed to provide neuroscientists at all levels with a conceptual and practical understanding of several of the most advanced techniques in molecular neuroscience. The course curriculum was divided into three sections: an extensive and up-to-date set of laboratory exercises, daily lectures covering the theoretical and practical aspects of the various methods used in the laboratory, and a series of evening research seminars. The informal and interactive evening lectures were given by leading molecular neuroscientists and served to illustrate the ways in which the various experimental approaches have been used to advance specific areas of neurobiology.

In this year's course, the laboratory portion included topics such as an introduction to the design and use of animal virus vectors in neurobiology; the use of small interfering RNAs (siRNAs) for regulating the

expression of specific genes in neurons; practical exercises in gene delivery systems including mammalian cell transfection protocols and single-cell electroporation techniques for targeted gene transfer in vivo; an introduction to overall strategies, use and design of BAC transgenic vectors; multiplex and whole-genome expression analyses using the most recent DNA microarray technologies (including labeled probe preparation, data analyses, mining, and interpretation); quantitative real-time RT-PCR analyses from small numbers of cells (RNA purification, PCR optimization, interpretation of results); single-cell PCR and cDNA library construction; and methods and application of RNA amplification (aRNA).

Each laboratory module was followed by comprehensive data analyses and interpretation, protocol troubleshooting, and suggestions for ways to improve or modify the existing technique. Finally, course participants were introduced to bioinformatics and a wide range of Internet resources that are available to molecular neuroscientists.

Speakers in the course included: Robert Darnell (HHMI/The Rockefeller University), Beverly Davidson (University of Iowa), Peter Hotez (George Washington University), Kenneth Kosik (Neuroscience Research Institute), Jeff Lichtman (Harvard University), Pierre-Marie Lledo (Pasteur Institute), Donald Lo (Duke Center for Drug Discovery), Ardem Patapoutian (The Scripps Research Institute), Peter Reinhart (Wyeth Reseach), Karel Svoboda (Janelia Farm Research Campus/HHMI), and George Yancopoulos (Regeneron Pharmaceuticals, Inc.).

This course was supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders & Stroke, and the Howard Hughes Medical Institute.

PARTICIPANTS

Arregui, L., M.S., Cinvestav, Mexico
Felsen, G., Ph.D., Cold Spring Harbor Laboratory
Fioravante, D., Ph.D., Harvard Medical School, Boston, Massachusetts
Geddie, M., B.S., Whitehead Institute for Biomedical Research, Cambridge, Massachusetts
Ghosh, J., Ph.D., University of Washington, Seattle
Hsu, C., B.A., Boston University School of Medicine, Massachusetts
Hundahl, C.A., Ph.D., University of Aarhus, Denmark
Jones, K., B.S., Univesity of California, San Diego, La Jolla
Koos, T., Ph.D., Rutgers University, Newark, New Jersey
Mayoral, S., B.S., Stanford University, California
Potter, G., Ph.D., University of California, San Francisco
Prescott, S., Ph.D., Salk Institute, La Jolla, California
Reyes-Colon, D., B.S., University of Puerto Rico, San Juan
Sequerra, E., M.Sc., Universidade Federal de Rio de Janeiro, Brazil
Tang, L., B.S., Johns Hopkins University School of Medicine, Baltimore, Maryland
Torbidoni, A., Lic., Facultad de Ciencias Biomedicas, Pilar, Argentina

SEMINARS

Darnell, R., HHMI/The Rockefeller University, New York: RNA-binding proteins and the regulation of neuronal synaptic plasticity.
Davidson, B., University of Iowa College of Medicine, Iowa City: RNA interference: Biological tool and therapeutic modality.
Eberwine, J., University of Pennsylvania Medical School, Philadelphia: Molecular biology of dendrite functioning—A few surprises... .
Hotez, P., George Washington University, Washington, D.C.: The neglected tropical diseases: The ancient afflictions of stigma and poverty.
Kosik, K., Neuroscience Research Institute, Santa Barbara, California: Neuronal microRNAs.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system.
Lledo, P.-M., Pasteur Institute, Paris, France: Wiring newborn neurons with old circuits.
Patapoutian, A., The Scripps Research Institute, Del Mar, California: The fifth sense: The chemistry and biology of temperature and pain detection.
Reinhart, P., Wyeth Research, Princeton, New Jersey: Developing disease modifying therapeutics for the treatment of Alzheimer's disease.
Svoboda, K., Janelia Farm Research Campus/HHMI, Ashburn, Virginia: Analysis of neuronal circuits.
Yancopoulos, G., Regeneron Pharmaceuticals, Inc., Tarrytown, New York: FO generation mice fully derived from gene-targeted embryonic stem cells.

Advanced Techniques in Plant Sciences

June 29–July 19

INSTRUCTORS **T. Brutnell,** Boyce Thompson Institute, Ithaca, New York
V. Irish, Yale University, New Haven, Connecticut
E. Kellogg, University of St. Louis, Missouri
J. Normanly, University of Massachusetts, Amherst

ASSISTANTS **L. Bai,** Cornell University, Ithaca, New York
K. Geuten, Yale University, New Haven, Connecticut
M. Lewandowski, University of Massachusetts, Amherst

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 was 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. These seminars included plant morphology and anatomy; plant development (such as development of flowers, leaves, male and female gametophytes, and roots); perception of light and photomorphogenesis; cell wall biosynthesis, function, and perception of hormones and application of research results to 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 to 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.

Invited speakers included Kenneth Birnbaum (New York University), Andy Bhattacharjee (Agilent), John Celenza (Boston University), Savithramma Dinesh-Kumar (Yale University), Richard Flavell (Ceres Inc.), Kevin Folta (University of Florida), Erich Grotewold (The Ohio State University), Stacey Harmer (University of California, Davis), David Jackson (Cold Spring Harbor Laboratory), Georg Jander (Boyce Thompson Institute, Cornell University), Nancy Kerk (Yale University), Cris Kuhlemeier (University of Bern), Thomas Nuhse (University of Manchester, United Kingdom), Torbert Rocheford (University of Illinois), Jocelyn Rose (Cornell University), Eric Schaller (Dartmouth University), Ian Sussex (Yale University), Peter Tiffin (University of Minnesota), and Marja Timmermans (Cold Spring Harbor Laboratory).

This course was supported with funds provided by the National Science Foundation.

PARTICIPANTS

Alcantara, J., Ph.D., SemBioSys Genetic Inc., Alberta, Canada
Bertea, C., Ph.D., University of Turin, Italy
Bokowiec, M., M.A., University of Virginia, Charlottesville
Burhans, D., Ph.D., Canisius College, Buffalo, New York
De Paoli, H., M.S., University of Sao Paulo, Ribeirao Preto, Brazil
Feinberg, P., B.S., New York University, New York
Matus, T., Ph.D. candidate, Pontificia Universidad Catolica de Chile, Santiago, Chile
Opanowicz, M., Ph.D., John Innes Centre, Norwich, United Kingdom
Rosler, J., Dipl., Justus Liebig University Giessen, Germany
Ryden, A.-M., M.Sc., Groningen University, The Netherlands
Sheldon, S., Ph.D., Middlebury College, Connecticut
Tamaru, H., Ph.D., Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria
Yoshida, Y., M.S., Kyoto University, Japan
Zepeda Jazo, A.I., M.S., University of Colima, Mexico

SEMINARS

Bhattacharjee, A., Agilent, Andover, Massachusetts: ChIP-chip data analysis.
Birnbaum, K., New York University, New York: Root development.
Callis, J., University of California, Davis: Protein turnover.
Celenza, J., Boston University, Massachusetts: Secondary metabolites: Glucosinolates.
Dinesh-Kumar, S., Yale University, New Haven, Connecticut: Plant pathogen interactions.
Flavell, R., Ceres, Inc., Thousand Oaks, California: Plant biomass and energy.
Folta, K., University of Florida, Miami: Light regulation.
Grotewold, E., University of Ohio, Columbus: Regulatory networks.
Irish, V., Yale University, New Haven, Connecticut: Reproductive development.
Jackson, D., Cold Spring Harbor Laboratory: Shoot meristem development.
Jander, G., Boyce Thompson Institute, Ithaca, New York: Quantitative genetics.
Kellogg, E., University of Missouri, St. Louis: Phylogenetics.
Kuhlemeier, C., University of Bern, Switzerland: Phyllotaxis.
Normanly, J., University of Massachusetts, Amherst: Metabolomics.
Nuhse, T., University of Manchester, United Kingdom: Proteomics.
Rocheford, T., University of Illinois, Urbana: Secondary metabolites, carotenoids.
Rose, J., Cornell University, Ithaca, New York: Cell walls and biofuels.
Schaller, G.E., Dartmouth College, Hanover, New Hampshire: Plant hormones.
Sussex, I., Yale University, New Haven, Connecticut: Introduction to plant structure.
Tiffin, P., University of Minnesota, Minneapolis: Evolution of pathogen resistance.
Timmermans, M., Cold Spring Harbor Laboratory: microRNA regulation.

Neurobiology of *Drosophila*

June 29–July 19

INSTRUCTORS **G. Bashaw,** University of Pennsylvania, Philadelphia
S. Waddell, University of Massachusetts Medical School, Worcester
B. Zhang, University of Oklahoma, Norman

ASSISTANTS **H. Bao,** University of Oklahoma, Norman
T. Evans, University of Pennsylvania Medical School, Philadelphia
M. Krashes, University of Massachusetts Medical School, Worcester
L. Yang, University of Pennsylvania, Philadelphia

This laboratory/lecture course was intended for researchers at all levels from beginning graduate students to established primary investigators who want to use *Drosophila* as an experimental system for nervous system investigation. The three-week course was divided into the study of development, physiology/function, and behavior. Daily seminars introduced students to a variety of research topics and developed those topics by including recent experimental contributions and outstanding questions in the field. Guest lecturers brought original preparations for viewing and discussion and direct laboratory exercises and experiments in their area of interest. The course provided students with hands-on experience using a variety of experimental preparations that were used in the investigation of current neurobiological questions.

The lectures and laboratories focused on both the development of the nervous system and its role in controlling larval and adult behaviors. In addition to an exposure to the molecular genetic approaches

available in *Drosophila*, students learned a variety of techniques including embryo in situ hybridization, labeling of identified neurons, electrophysiology (intracellular recording and patch clamping), optical imaging of neuronal activity, and the analysis of larval and adult behavior. Collectively, the course provided a comprehensive and practical introduction to modern experimental methods for studying the *Drosophila* nervous system.

This year's lecturers included Heather Broihier (Case Western Reserve University), Vivian Budnik (University of Massachusetts), Sarah Certel (Harvard Medical School), Josh Dubnau (Cold Spring Harbor Laboratory), Aki Ejima (Brandeis University), David Featherstone (University of Illinois at Chicago), Fen-Biao Gao (The Gladstone Institutes at UCSF), Leslie Griffith (Brandeis University), Gregory Jefferis (University of Cambridge), Maurice Kernan (Stony Brook University), Haig Keshishian (Yale University), Edward Kravitz (Harvard Medical School), Adelaine Leung (MRC-Laboratory of Molecular Biology), Mala Murthy (CalTech), Amita Sehgal (HHMI/University of Pennsylvania), Ping Shen (University of Georgia), Stephan Sigrist (University of Wurzburg), Bryan Stewart (University of Toronto), Susan Tsunoda (Boston University), Glenn Turner (Cold Spring Harbor Laboratory), Bruno van Swinderen (Neurosciences Institute), and Jie Xu (The University of Georgia).

This course ws supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders & Stroke, and the Howard Hughes Medical Institute.

PARTICIPANTS

Altman, J., B.A., University of Washington, Seattle
Behnia, R., Ph.D., New York University, New York
Berry, J., B.S., Baylor College of Medicine, Houston, Texas
Brierley, D., B.Sc., King's College London, United Kingdom
Chiang, A., M.S., National Centre for Biological Sciences, Bangalore, India
Friedman, E., M.D., University of Pennsylvania/HHMI, Philadelphia
Holt, M., Ph.D., Max-Planck Institute for Biophysical Chemistry, Goettingen, Germany
Im, S.H., M.S., Washington University School of Medicine, St. Louis, Missouri
Imad, M., Ph.D., University of Arizona, Tucscon
Koch, M., Ph.D., VIB and Kuleuven, Leuven, Belgium
Loya, C., B.S., Harvard Medical School, Boston, Massachusetts
Seay, D., Ph.D., The Rockefeller University, New York

SEMINARS

Bashaw, G., University of Pennsylvania, Philadelphia: Axon guidance. Genetics 101.
Brohier, H., Case Western Reserve, Cleveland, Ohio: Neuronal identity.
Budnik, V., University of Masschusetts, Worcester: NMJ development.
Featherstone, D., University of Illinois, Chicago: Development and regulation of glutamatergic signaling.
Freeman, M., University of Massachusetts, Worcester: Glial development and Fn. Bioinformatics 101.
Gao, F.B., University of California, San Francisco: Dendrite morphogenesis.
Griffith, L. and Ejima, A., Brandeis University, Waltham, Massachusetts: Courtship.
Jefferis, G., Cambridge University, Cambridge, United Kingdom: Olfactory system development. Mosaic genetic techniques.
Kerman, M., Stony Brook University, New York: Mechanotransducers and mechanotransduction.
Keshishian, H., Yale University, New Haven, Connecticut: Neuronal activity and synaptic plasticity.
Kravitz, E. and Certel, S., Harvard Medical School, Boston, Massachusetts: Aggression.
Sehgal, A., University of Pennsylvania, Philadelphia: Circadian rhythm and sleep.
Shen, P., University of Georgia, Athens: Feeding behavior.
Sigrist, S., European Neuroscience Institute, Gottingen, Germany: Shedding light on assembly of synapse structure and function.
Stewart, B., University of Toronto, Canada: Synaptic vesicle exocytosis and beyond. Electrophysiology 101.
Tsunoda, S., Boston University, Massachusetts: Phototransduction and the dynamic nature of signaling components in the eye.
Turner, G., Cold Spring Harbor Laboratory, and Murthy, M., CalTech, Pasadena, California: Neural coding in the fly brain: In vivo whole-cell patch-clamp recording methods.
Van Swinderen, B., Neuroscience Institute, San Diego, California: Visual learning/attention.
Waddell, S., University of Massachusetts Medical School, Worcester: Introduction.
Waddell, S., University of Massachusetts Medical School, Worcester, and Dubnau, J., Cold Spring Harbor Laboratory: Olfactory learning and memory.
Zhang, B., University of Oklahoma, Norman: Synaptic vesicle recycling.

Structure, Function, and Development of the Visual System

July 6–19

INSTRUCTORS **B. Chapman,** University of California, Davis
W.M. Usrey, University of California, Davis

ASSISTANT **C. Speer,** University of California, Davis

This lecture/discussion course explored the functional organization and development of the visual system as revealed by the use of a variety of anatomical, physiological, and behavioral methods. It was designed for graduate students and more advanced researchers who wish to gain a basic understanding of the biological basis for vision and to share in the excitement of the latest developments in this field. Topics included phototransduction and neural processing in the retina; functional architecture of striate cortex; cellular basis of cortical receptive field properties; the anatomy, physiology, and perceptual significance of parallel pathways; functional parcellation of extrastriate cortex; the role of patterned neuronal activity in the development of central visual pathways; and molecular mechanisms of development and plasticity in the visual system.

Speakers in the course included Katja Brose (Cell Press), E.J. Chichilnisky (Salk Institute for Biological Studies), Dennis Dacey (University of Washington), James DiCarlo (Massachusetts Institute of Technology), Marla Feller (University of California, San Diego), David Fitzpatrick (Duke University), Bill Guido (Virginia Commonwealth University), Michael Hawken (New York University), Judith Hirsch (University of

Southern California), Nancy Kanwisher (Massachusetts Institute of Technology), Rich Krauzlis (Salk Institute for Biological Studies), Tirin Moore (Stanford University), Tony Movshon (New York University), Jay Neitz (Medical College of Wisconsin), Tatiana Pasternak (University of Rochester), Clay Reid (Harvard Medical School), Ed Ruthazer (McGill University), Mike Shalden (University of Washington), Murray Sherman (University of Chicago), Lee Stone (NASA Ames Research Center), and David Williams (University of Rochester).

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Caplovitz, G., B.S., Dartmouth College, Enfield, New Hampshire
Federer, F., B.S., University of Utah, Salt Lake City
Goris, R., B.S., University of Leuven, Belgium
Henriksson, L., B.S., Helsinki University of Technology, Espoo, Finland
Hussar, C., B.S., University of Rochester, New York
Jiang, F., B.S., University of Texas, Dallas
Karlen, S., B.S., University of California, Davis
Kurki, I., B.S., University of Helsinki, Finland
Law, J., B.S., University of Edinburgh, United Kingdom
MacKenzie, K., B.S., Brandeis University, Waltham, Massachusetts
Mauck, M., B.S., Medical College of Wisconsin, Milwaukee
Morhardt, D., B.S., Virginia Commonwealth University, Richmond
Muhammad, R., B.S., Massachusetts Institute of Technology, Cambridge
Olkkonen, M., B.S., Justus-Leibig University, Giessen, Germany
Rochefort, N., Ph.D., Institute of Neuroscience, Munich, Germany
Schmidt, A., B.S., Max-Planck Institute for Brain Research, Frankfurt, Germany
Shushruth, S., B.S., University of Utah, Salt Lake City
Soto-Sanchez, C., Ph.D., University of Southern California, Los Angeles
Swearington, J., B.S., Medical University of South Carolina, Charleston
Wang, C., Ph.D., Washington University, St. Louis, Missouri
Wang, X., B.S., University of Southern California, Los Angeles
Wei, H., B.S., University of Louisville, Kentucky
Weng, C., B.S., State University of New York, College of Optometry

SEMINARS

Brose, K., Cell Press, Cambridge, Massachusetts: Behind the scenes of the review process at neuron.
Chapman, B., University of California, Davis: How the visual system got its stripes.
Chichlilnisky, E.J., The Salk Institute, La Jolla, California: Ensemble coding of visual information in primate retina.
Dacey, D., University of Washington, Seattle: Parallel visual pathways and retinal microcircuits.
DiCarlo, J., Massachusetts Institute of Technology, Cambridge: Neuronal representation of the visual world: What is the ventral visual stream doing?
Feller, M., University of California, San Diego: The mechanisms and function of retinal waves.
Fitzpatrick, D., Duke University, Durham, North Carolina: The functional architecture of cortical circuits.
Guido, W., Virginia Commonwealth University, Richmond: Development of the retinogeniculate pathway: Structure, function, and underlying mechanisms.
Hawken, M., New York University, New York: Structure and function in macaque monkey V1.
Hirsch, J., University of Southern California, Los Angeles: Comparing thalamus with cortex: Conserved and unique features of neural circuits that build visual receptive fields.
Kanwisher, N., Massachusetts Institute of Technology, Cambridge: fMRI investigations of the functional organization of the ventral visual pathway.
Krauzlis, R., The Salk Institute, San Diego, California: Coordination of voluntary eye movements.
Moore, T., Stanford University, California: Neural control of visual spatial attention.
Movshon, A., New York University, New York: Visual response properties of MT neurons.
Neitz, J., Medical College of Wisconsin, Milwaukee: Photopigments and photoreceptors: Their evolution and their role in vision.
Oberdorfer, M., National Institutes of Health, Bethesda, Maryland: NEI/NIH support for vision research.
Pasternak, T., University of Rochester, New York: Responses to behaviorally relevant visual motion in primate cortex.
Reid, C., Harvard Medical School, Boston, Massachusetts: Imaging neurons in the brain.
Ruthazer, E., McGill University, Montreal, Canada: Visual system development in fish and frogs.
Shadlen, M., University of Washington, Seattle: A vision of decision-making.
Sherman, M., University of Chicago, Illinois: Role of thalamus in the flow of information to cortex.
Steinmetz, M., Center for Scientific Review/NIH, Bethesda, Maryland: Peer review at NIH.
Stone, L., NASA Ames Research Center, Moffett Field, California: Visual processing for perception and oculomotor action.
Usrey, M., University of California, Davis: Visual processing in the retinogeniculocortical pathway.
Williams, D., University of Rochester, New York: The limits of human vision.

Eukaryotic Gene Expression

July 18–August 7

INSTRUCTORS **M. Bulger,** University of Rochester School of Medicine, Rochester, New York
T. Oelgeschläger, Marie Curie Research Institute, Oxted, United Kingdom
A. Shilatifard, Stowers Institute for Medical Research, Kansas City, Missouri

ASSISTANTS **V. Caputo,** Marie Curie Research Institute, Oxted, United Kingdom
G. Fromm, University of Rochester, Rochester, New York
P.F. Wang, University of Nebraska Medical Center, Omaha

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 employed in the field. Emphasis was placed both on in vitro and in vivo protein–DNA interactions and on novel methodologies to study gene regulation. Students made nuclear extracts, performed in vitro transcription reactions, and measured RNA levels using primer extension. Characterizations of the DNA-binding properties of site-specific transcription factors were carried out using electrophoretic mobility shift and DNase I footprinting assays. In addition, students learned techniques for the assembly and analysis of chromatin in vitro, including transcription assays, chromatin footprinting, and chromatin remodeling assays.

During the past few years, the gene regulation field has developed in vivo approaches to study gene regulation. Students were exposed to the chromatin immunoprecipitation technique. They used RNAi for specific knockdown experiments in mammalian cells. In addition, determining cellular gene expression profiles has been accelerated tremendously by DNA microarray technology. Students received hands-on training in performing and interpreting results from DNA microarrays.

Experience with basic recombinant DNA techniques was a prerequisite for admission to this course. Lectures by the instructors covered the current status of the gene expression field, theoretical aspects of the methodology, and broader issues regarding strategies for investigating the regulation of gene expression in eukaryotes. Guest lecturers discussed contemporary problems in eukaryotic gene regulation and technical approaches to their solution.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Camargo, M., Ph.D., University of Sao Paulo, Brazil
Hsu, C.-P., Ph.D., Academia Sinica, Taipei, Taiwan
Johnson, M., Ph.D., Teagasc, Fermoy, Republic of Ireland
Karouzakis, E., M.S., University of Hospital Zurich, Switzerland
Kimura, M., Ph.D., Gifu University School of Medicine, Japan
Kurata, H., Ph.D., Washington University School of Medicine, St. Louis, Missouri
Mannikarottu, A., Ph.D., Albany College of Pharmacy, New York
Monrad, A., M.S., University of Copenhagen, Denmark
Prucca, C., M.Sc., Mercedes & Martin Ferreyra Medical Research, Cordoba, Argentina
Puliafito, A., Ph.D., University of California, Santa Barbara
Shilov, B., Ph.D., Siberian State Medical University, Tomsk, Russia
Sonderby, I., M.S., Copenhagen University, Denmark
Stoytcheva, Z., Ph.D., University of Hawaii, Honolulu
Teixeira, C., Ph.D., New York University, New York
White, A., M.S., Rutgers University, Piscataway, New Jersey
Yip, S.P., Ph.D., The Hong Kong Polytechnic University, China

SEMINARS

Becker, P., Ludwig Maxmilians Universität, München, Germany: Dosage compensation in *Drosophia:* Beginning and end of generalization.
Berger, S., The Wistar Institute, Philadelphia, Pennsylvania: Histone modifications and gene expression.
Bickmore, W., Western General Hospital, Edinburgh, United Kingdom: Linking nuclear organization and gene expression.
Bulger, M., University of Rochester, New York: Patterns of histone modification and gene expression erythroid cells.
Buratowski, S., Harvard Medical School, Boston, Massachusetts: The RNA polymerase II CTD mediates multiple mechanisms for suppressing cryptic transcription.
Conaway, J., Stowers Institute for Medical Research, Kansas City, Missouri: The basal transcription machinery: Biochemistry of a multicomponent system.
Fraser, P., The Babraham Institute, Cambridge, United Kingdom: Higher-order chromatin/chromosome conformations, transcription, and nuclear organization of the genome.
Hahn, S., Fred Hutchinson Cancer Research Center, Seattle, Washington: Transcription initiation and activation.
Levine, M., University of California, Berkeley: Gradient thresholds in the *Drosophila* embryo and heart cell migration in the ciona tadpole.
Oelgeschläger, T., Marie Curie Research Institute, Surrey, United Kingdom: Role of the core promoter in the regulation of RNA polymerase II transcription.
Roeder, R., The Rockefeller University, New York: Transcription by RNA polymerase II.
Schultz, P., Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France: Structural studies of transcription complexes by electron microscopy and tomography.
Shiekhattar, R., The Wistar Institute, Philadelphia, Pennsylvania: The RNAi machinery.
Shilatifard, A., Stowers Institute for Medical Research, Kansas City, Missouri: Transcription elongation control, histone methylation, and human leukemia.
Tora, L., Institut de Genetique et de Biologie Moleculaire et Cellulaire, Strasbourg, France: TFTC/STAGA HAT COMPLEX links stress response, histone, H2B deubiquitination-dependent chromatin remodeling, and transcription activation.
Tsukiyama, T., Fred Hutchinson Cancer Research Center, Seattle, Washington: Chromatin remodeling determines transcriptional orientation.
Workman, J., Stowers Institute for Medical Research, Kansas City, Missouri: Protein complexes that modify chromatin for transcription.

Biology of Memory

July 22–August 4

INSTRUCTORS **K. Martin,** University of California, Los Angeles
J. Raymond, Stanford University, California

This lecture course provided an introduction to cellular, molecular, and systems approaches to learning and memory. It was suited for graduate students and postdoctoral fellows in molecular biology, neurobiology, and psychology, as well as research workers who are interested in an introduction to this field. The course covered topics ranging from behavioral considerations of learning and memory to gene regulation in the nervous system. The lectures provided an intensive coverage of modern behavioral studies of learning and memory, the cell and molecular biology of neuronal plasticity, cellular and molecular mechanisms of simple forms of learning and memory, and systems approaches to learning in vertebrates and humans. Lectures were complemented by exercises in which students worked in small groups with lecturers to discuss topical issues in learning and memory, to evaluate recent studies, and to identify and formulate new research questions and approaches. The course was thus designed not only to introduce students to the field of learning and memory, but also to provide an intellectual framework upon which future studies can be built.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Borlikova, G., B.S., Okinawa Institute of Science & Technology, Uruma, Japan
Chen, D., B.S., Mount Sinai School of Medicine, New York
Ch'ng, T.H., Ph.D., University of California, Los Angeles
De Pasquale, R., B.S., Scuola Normale Superiore, Pisa, Italy
Dieguez, Jr., D., Ph.D., Boston University, Massachusetts
Hornsberger, M., B.S., McGill University, Montreal, Canada
Jeffrey, R., B.S., University of California, Los Angeles
Jorgensen, A., B.S., Lundbeck University, Valby, Denmark
Kennedy, P., B.S., Mount Sinai School of Medicine, New York
King, S., B.S., University of Virginia, Richmond
Kinzer-Ursem, T., B.S., California Institute of Technology, Pasadena
Lazaro-Munoz, G., B.S., New York University, New York
Miles, L., B.S., Emory University, Atlanta, Georgia
Moreira, F., Ph.D., Johannes Gutenberg University, Mainz, Germany
Nguyen-Vu, T.D.B., B.S., Stanford Medical School, California
Shin, S.-L., Ph.D., Stanford University, California
Shobe, J., Ph.D., University of California, Irvine
Shrager, Y., B.S., University of California, San Diego
Taylor, A., Ph.D., California Institute of Technology, Pasadena
Ye, X., B.S., University of California, Irvine

SEMINARS

Bargmann, C., The Rockefeller University, New York: *C. elegans*.
Bargmann, C., The Rockefeller University, New York, and Tully, T., Cold Spring Harbor Laboratory: Model genetic systems for studying memory.
Brody, C., Princeton University, New Jersey: Computational approaches to learning and memory.
Byrne, J., University of Texas–Houston Medical School, Houston: Aplysia as a model system for studying memory.
Dan, Y., University of California, Berkeley: Spike-timing-dependent plasticity.
Eichenbaum, H., Boston University, Massachusetts: Hippocampal-mediated memories.
Holland, P., Johns Hopkins University, Baltimore, Maryland: Learning theory.
Kennedy, M., California Institute of Technology, Pasadena: Biochemistry of synaptic plasticity.
LeDoux, J., New York University, New York: Amygdala-mediated memories.
Madison, D., Stanford University School of Medicine, California: Synaptic plasticity, LTP, and LTD.
Martin, K., University of California, Los Angeles: Cell biology of learning-related plasticity.
Raymond, J., Stanford University, California: Cerebellar-mediated memories.
Silva, A., University of California, Los Angeles: Molecular cognition: Animal models of cognitive disorders.
Squire, L., University of California, San Diego: Historical overview of memory research. Studies of memory in humans.
Sur, M., Massachusetts Institute of Technology, Cambridge: Map plasticity in the cortex.
Tully, T., Cold Spring Harbor Laboratory: *Drosophila*.

Imaging Structure and Function in the Nervous System

July 24–August 13

INSTRUCTORS **F. Engert,** Harvard University, Cambridge, Massachusetts
M. Hubener, Max-Planck Institute of Neurobiology, Martinsried, Germany
D. Kleinfeld, University of California, San Diego, La Jolla
J. Waters, Northwestern University, Chicago, Illinois

ASSISTANTS **F. Albineau,** Harvard University, Cambridge, Massachusetts
A. Kampff, Harvard University, East Aurora, New York
T. Mrsic-Flogel, University College London, United Kingdom
V. Nagerl, Max-Planck Institute of Neurobiology, Munich, Germany
M. Rhiannon, University of Oxford, United Kingdom
A. Trevelyan, University of Newcastle, United Kingdom
P. Tsai, University of California, San Diego, La Jolla
J. Tyler, Arizona State University, Tempe
R. Vislay, Harvard University, Cambridge, Massachusetts

Advances in light microscopy and digital image processing, and the development of a variety of powerful fluorescent probes, present expanding opportunities for investigating the nervous system from synaptic spines to networks in the brain. This intensive laboratory and lecture course provided participants with the theoretical and practical knowledge to utilize emerging imaging technologies.

The primary emphasis of the course was on vital light microscopy. Students learned the principles of light microscopy, as well as use of different types of electronic cameras, laser-scanning systems, func-

tional fluorophores, delivery techniques, and digital image-processing software. In addition to transmitted light microscopy for viewing cellular motility, the course examined a variety of molecular probes of cell function, including calcium-sensitive dyes, voltage-sensitive dyes, photo-activated ("caged") compounds, and exocytosis tracers. Issues arising in the combination of imaging with electrophysiological methods were covered. Particular weight was given to multi-photon 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. Participants had a strong background in the neurosciences or in cell biology.

Lecturers in the course included Hollis Cline (Cold Spring Harbor Laboratory), Karl Deisseroth (Stanford University), David DiGregorio (Universite Paris 5), Oliver Griesbeck (Max-Planck Institute of Neurobiology), Kalanit Grill-Spector (Stanford University), Fred Lanni (Carnegie-Mellon University), Jeff Lichtman (Harvard University), Jerome Mertz (Boston University), Tom Mrsic-Flogel (University College London), Venkatesh Murthy (Harvard University), Valentin Nagerl (Max-Planck Institute of Neurobiology), Claudia Racca (Newcastle University), Botond Roska (FMI), Petra Schwille (Tu Dresden, Germany), Karel Svoboda (Janelia Farm Research Campus/HHMI), Karen Willig (Max-Planck Institute for Biological Chemistry), and Xiaowei Zhuang (Harvard University).

This course was supported with funds provided by the National Institute of Mental Health, the National Institute of Neurological Disorders & Stroke, and the Howard Hughes Medical Institute.

PARTICIPANTS

Brennan, K,, Jr., M.D., University of California, Los Angeles
Chen, B.-S., Ph.D., NINDS/NIH, Bethesda, Maryland
Dhawale, A., B.Sc., National Centre for Biological Sciences, Bangalore, India
Ewers, H., M.S., ETH Zurich, Switzerland
Helling, I., Dipl., Max-Planck Institute for Neurobiology, Munich, Germany
Histed, M., Ph.D., Harvard Medical School, Boston, Massachusetts
Kim, J.H., Ph.D., Oregon Health & Science University, Portland
Li, B., Ph.D., Cold Spring Harbor Laboratory
Pelletier, J., Ph.D., Universite de Montreal, Canada
Santhakumar, V., Ph.D., University of California, Los Angeles
Scimemi, A., Ph.D., National Institutes of Health, Bethesda, Maryland
Wu, L.-J., Ph.D., University of Toronto, Canada

SEMINARS

Cline, H., Cold Spring Harbor Laboratory: Tadpoles rock.
Deisseroth, K., Stanford University, California: Light-triggered activation/silencing.
DiGregorio, D., Universite Paris 5, Paris, France: Flash photolysis.
Engert, F., Harvard University, Cambridge, Massachusetts: Home-brew 2-photon overview.
Engert, F. and Kampff, A., Harvard University, Cambridge, Massachusetts: Shot noise, ImageJ, CCD cameras, PMTs.
Griesbeck, O., Max-Planck Institute of Neurobiology, Martinsried, Germany: Geneticially encoded sensors.
Grill-Spector, K., Stanford University, California: fMRI.
Hubener, M., Max-Planck Institute of Neurobiology, Martinsried, Germany: Intrinsic imaging.
Kleinfeld, D., University of California, San Diego, La Jolla: De-noising 1. De-noising 2. Blood flow. Voltage-sensitive dyes.
Lanni, F., Carnegie-Mellon University, Pittsburgh, Pennsylvania: Basic microscopy. Basic microscopy diffraction theory. Light sources. DIC, Dot tube, Apotome, Demo.
Lichtman, J., Harvard University, Cambridge, Massachusetts: Confocal microscopy.
Mertz, J., Boston University, Boston, Massachusetts: Nonlinear techniques.
Mrsic-Flogel, T., University College London, United Kingdom: Ca bulk loading in vivo.
Murthy, V., Harvard University, Cambridge, Massachusetts: Synaptofluorins.
Naegerl, V., Max-Planck Institute of Neurobiology, Munich, Germany: Bring in da noise: 2p nuggets and applications.
Racca, C., Newcastle University, Newcastle upon Tyne, United Kingdom: Electron microscopy.
Roska, B., FMI, Basil, Switzerland: Viruses.
Schwille, P., Tu Dresden, Saxony, Germany: FCS
Svoboda, K., Janelia Farm Research Campus/HHMI, Ashburn, Virginia: Basics of 2p imaging. Advanced 2p FRET< FLIM, 2p uncaging. 2p and Serial EM.
Tsai, P., University California, San Diego, La Jolla: Basic optical design. Basic design scanning: Fluorescence.
Waters, J., Northwestern University, Evanston, Illinois: Organic Ca indicators. In vivo Ca imaging.
Willig, K., Max-Planck Institute of Neurobiology, Goettingen, Germany: Breaking the resolution limit.
Zhuang, X., Harvard University, Cambridge, Massachusetts: STORM.

Yeast Genetics and Genomics

July 24–August 13

INSTRUCTORS **B. Errede,** University of North Carolina, Chapel Hill
F. Luca, University of Pennsylvania, Philadelphia
M. Whiteway, National Research Council of Canada, Montreal

ASSISTANTS **V. Kuravi,** University of Pennsylvania, Philadelphia
H. Lavoie, McGill University, Montreal, Quebec, Canada
M. Mangos, National Research Council of Canada, Montreal, Quebec

This course was a modern state-of-the-art laboratory course designed to teach the students the full repertoire of genetic approaches needed to dissect complex problems in the yeast *Saccharomyces cerevisiae*. Combinations of classical genetic approaches were emphasized, including the isolation and characterization of mutants, tetrad analysis, complementation, and mitotic recombination. Molecular genetic techniques included various types of yeast transformations, gene replacement with plasmids and PCR, construction and analysis of gene fusions, and generation of mutations in cloned genes.

Students used classical and molecular approaches to gain experience in identifying and interpreting various kinds of genetic interactions including suppression and synthetic lethality. They were immersed in yeast genomics and performed and interpreted experiments with DNA arrays. The participants 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 vari-

ous subcellular organelles. Lectures on fundamental aspects of yeast genetics were presented along with seminars given by outside speakers on topics of current interest.

This year's speakers included David Amberg (SUNY Upstate Medical University), Angelika Amon (Massachusetts Institute of Technology), Judith Berman (University of Minnesota), Charlie Boone (University of Toronto), James Broach (Princeton University), Dan Burke (University of Virginia), Dean Dawson (Tufts University), Kara Dolinski (Princeton University), Daniel Finley (Harvard Medical School), Daniel Lew (Duke University), Andrew Murray (Harvard University), Liza Pon (Columbia University), Jeffrey Strathern (National Cancer Institute), and Lorraine Symington (Columbia University College of Physicians and Surgeons).

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Basu, A., B.S., The Rockefeller University, New York
Behar, M., Lic., The University of North Carolina, Chapel Hill
De Smet, C., M.S., Utrecht University, The Netherlands
Durand, D., Ph.D., Carnegie-Mellon University, Pittsburgh, Pennsylvania
Im, Y.J., Ph.D., NIDDK/NIH, Bethesda, Maryland
Iofik, A., M.S., University of Tartu, Estonia
Ketel, C., Ph.D., University of Minnesota, Minneapolis
Kinkhabwala, A., Ph.D., Max-Planck Institute for Molecular Physiology, Dortmund, Germany
Rest, J., Ph.D., University of Chicago, Illinois
Reyes, A.C., Ph.D., Göteborg University, Sweden
Reynolds, N., M.S., The Ohio State University, Columbus
Rocha, W., Ph.D., University of Montreal, Canada
Rydén, A.-M., M.Sc., University of Groningen, The Netherlands
Sankovich, S., B.S., CSIRO, Parkville, Australia
Sanyal, S., M.S., Cornell/Rockefeller/Sloan-Kettering, New York
Sigurgislason, H., M.S., University of Iceland, Reykjavik

SEMINARS

Amberg, D., SUNY Upstate Medical University, Syracuse, New York: Actin up in the Amberg lab.
Amon, A., Massachusetts Institute of Technology, Cambridge: Causes and consequences of aneuploidy.
Berman, J., University of Minnesota, Minneapolis: Genomics and genome instability in *Candida albicans*.
Boone, C., University of Toronto, Canada: Large-scale mapping of genetic and chemical-genetic interactions in yeast.
Broach, J., Princeton University, New Jersey: Nutrient sensing and information processing in yeast.
Burke, D., University of Virginia, Charlottesville: Spindle checkpoints.
Dawson, D., Oklahoma Medical Research Foundation, Oklahoma City: Meiotic centromere coupling: Holding chromosomes together to help them fall apart.
Finley, D., Harvard Medical School, Boston, Massachusetts: The ubiquitin-proteasome system.
Lew, D., Duke University, Durham, North Carolina: Cell polarity and cell cycle control.
Murray, A., Harvard University, Cambridge, Massachusetts: How yeast cells find a mate.
Pon, L., Columbia University, New York: Mitochondrial motility and inheritance in budding yeast: Lessons from the nucleus.
Strathern, J., National Cancer Institute, Frederick, Maryland: The fidelity of transcription.
Symington, L., Columbia University College of Physicians and Surgeons, New York: Mechanisms of homologous recombination.

Cellular Biology of Addiction

August 7–13

INSTRUCTORS **C. Evans,** University of California, Los Angeles
M. Von Zastrow, University of California, San Francisco

ASSISTANTS **C. Bryant,** University of Chicago, Illinois
M. Puthenveedu, University of California, San Francisco

Drug addiction is the most costly neuropsychiatric disorder faced by our nation. Acute and repeated exposure to drugs produces neuroadaption and long-term memory of the experience, but the cellular and molecular processes involved are only partially understood. The primary objective of the workshop was to provide an intense dialogue of the fundamentals, state-of-the-art advances and major gaps in the cell, and molecular biology of drug addiction.Targeted to new or experienced investigators, the workshop combined formal presentations and informal discussions to convey the merits and excitement of cellular and molecular approaches to drug addiction research.

With the advent of genomics and proteomics, an extraordinary opportunity now exists to develop comprehensive models of neuroadaptative processes fundamental to addiction, withdrawal, craving, and relapse to drug use and to brain function, in general. A range of disciplines and topics were represented, including noninvasive brain imaging to identify drug targets and adaptive processes; neuroadaptative processes at the molecular and cellular level, neural networks and their modulation, the relevance of genotype to susceptibility and drug response; tolerance and adaptation at the cellular level; and approaches to exploiting the daunting volume generated by neuroinformatics.

This workshop provided an integrated view of current and novel research on neuroadaptive responses to addiction, fostered discussion on collaboration and integration, and provided critical information needed to construct a model of addiction as a disease and novel molecular targets for biological treatments. Beyond the plane of scientific endeavor, the information is vital for formulating public policy and for enlightening the public on the neurobiological consequences of drug use and addiction. The proposed workshop was designed to generate interest in this level of analysis, open conduits for collaborations, and present novel routes to investigating the neurobiology of addictive drugs.

Speakers in the course included Huda Akil (University of Michigan), George Augustine (Duke University Medical Center), Wade Berrentini (University of Pennsylvania), Lakshmi Devi (Mount Sinai School of Medicine), Robert Edwards (University of California, San Francisco), David Goldman (NIAAA/LNG), Marin Iguchi (University of California, Los Angeles), Peter Kalivas (Medical University of South Carolina), Brigitte Kieffer (Institute de Genetique et de Biologie Moleculaire et Cellulaire, France), George Koob (Scripps Research Institute), Mary Jean Kreek (The Rockefeller University), Angus Nairn (Yale University School of Medicine), Eric Nestler (University of Texas Southwestern, Dallas), Marina Picciotto (Yale University), John Pintar (University of Medicine & Dentristry of New Jersey), Michael Rosenfeld (University of California, San Diego), Wolfgang Sadee (The Ohio State University), John Williams (Vollum Institute), Robert Williams (University of Tennessee), Renping Zhou (Rutgers University College of Pharmacy), and Jon-Kar Zubeita (University of Michigan).

This course was supported with funds provided by the National Institute of Drug Abuse.

PARTICIPANTS

Avena, N., Ph.D., The Rockefeller University, New York
Bland, S., Ph.D., University of Colorado,
Chen, X., Ph.D., Virginia Commonwealth University
Corlett, P., Ph.D., Cambridge University, United Kingdom
Culbertson, C., B.S., University of California, Los Angeles
Duke, A., B.S., Harvard Medical School,
Frantz, K., Ph.D., Georgia State University
Garcia-Fuster, J., Ph.D., University of Michigan
Gaval, M., B.S., Emory University
Hostetler, C., B.S., University of California, Davis
Nair, S., Ph.D., National Institute on Drug Abuse/NIH, Bethesda, Maryland
Riley, B., Ph.D., Virginia Commonwealth University
Rothwell, P., B.S., University of Minnesota
Sable, H., Ph.D., University of Illinois, Urbana-Champaign
Tanabe, J., M.D., University of Colorado Health Sciences Center
Tidgewell, K., B.S., University of Iowa
Wallace, M., Ph.D., University of California, San Francisco
Wallach, M., Ph.D., Oregon Health & Science University
Wargelius, H., B.S., Uppsala University, Sweden
Williams, T., B.S., Weill Medical College of Cornell University
Yang, B.-Z., Ph.D., Yale University, New Haven, Connecticut
Yaragudri, V., Ph.D., Nathan Kline Institute for Psychiatric Research
Yu, J., B.S., University of California, San Francisco
Zhu, J., Ph.D., University of South Carolina

SEMINARS

Akil, H., University of Michigan, Ann Arbor: Vulnerability to drug abuse: Genetics and neuroplasticity.
Augustine, G., Duke University, Durham, North Carolina: Synapsins: Roles in synaptic vesicle trafficking and behavioral responses to psychotropic drugs.
Berrentini, W., University of Pennsylvania, Philadelphia: Whole-genome association studies of nicotine dependence.
Devi, L., Mount Sinai School of Medicine, New York: Heterodimerization of mu and delta opioid receptors: A role in modulating analgesia and addiction.
Edwards, R., University of California, San Francisco: The regulation of monoamine transporters.
Evans, C. and Von Zastrow, M., University of California, San Francisco: Introduction and course overview.
Goldman, D., NIH/NIAAA, Rockville, Maryland: Functional genetic variation addiction vulnerability via stress resiliency.
Iguchi, M., University of California, Los Angeles: Drug abuse and public policy: Making science matter.
Kalivas, P., Medical University of South Carolina, Mount Pleasant: Can we forget to be addicted?
Kieffer, B., Institute de Genetique et de Biologie Moleculaire et Cellulaire, Illkirch, France: Genetic approaches to study opioid receptor function.
Koob, G., Scripps Research Institute, La Jolla, California: Neurocircuitry of addiction: From reward to antireward.
Kreek, M.-J., The Rockefeller University, New York: Endorphins, gene polymorphisms, stress responsivity, and specific addictions: Selected topics.
Nairn, A., Yale University, New Haven, Connecticut: Dopamine-regulated signal transduction mechanisms and the actions of psychostimulants.
Nestler, E., University of Texas Southwestern, Dallas: Molecular mechanisms of drug addiction.
Picciotto, M., Yale University, New Haven, Connecticut: Molecular mechanisms underlying nicotine addiction.
Pintar, J., University of Medicine & Dentistry of New Jersey, Piscataway: Genetic studies of opiod system function.
Pollock, J., National Institute on Drug Abuse/NIH/DHHS, Bethesda, Maryland: Everything you wanted to know about funding at NIH but you didn't know who or what to ask.
Sadee, W., Ohio State University, Columbus: Searching for polymorphisms that affect gene expression and mRNA processing.
Williams, J., Vollum Institute/Oregon Health Sciences University, Portland: Opioids actions on single neurons and synaptic connections.
Williams, R., University of Tennessee, Memphis: Gene expression networks in the mouse brain and their linkage to addiction biology.
Zhou, R.P., Rutgers University, Piscataway, New Jersey: Development of the midbrain dopaminergic pathways.
Zubeita, J.-K., University of Michigan, Ann Arbor: Neurochemical measures of stress and reward response circuitry in the human brain.

C. elegans

August 11–26

INSTRUCTORS **S. Ahmed,** University of North Carolina, Chapel Hill
M. de Bono, MRC/Cambridge, United Kingdom
A. Desai, Ludwig Institute for Cancer Research, La Jolla, California

ASSISTANTS **A. Couto,** MRC-Laboratory of Molecular Biology, Cambridge, United Kingdom
A. Dammermann, University of California, San Diego
L. Shtessel, University of North Carolina, Chapel Hill

This course was designed to familiarize investigators with *C. elegans* as an experimental system, with an emphasis on both classical genetic analysis and reverse genetic approaches. A major goal was to teach students how to successfully exploit the information generated by the C. elegans genome project. The course was suited for those who have a current training in molecular biology and some knowledge of genetics but 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.

Speakers in the course included Andrew Dillin (The Salk Institute for Biological Studies), Monica Driscoll (Rutgers, The State University of New Jersey), Marie-Anne Felix (Institut Jacques Monod, CNRS), Andrew Fraser (The Wellcome Trust Sanger Institute), Barth Grant (Rutgers, The State University of New Jersey), Jonathan Hodgkin (University of Oxford), Geraldine Seydoux (The Johns Hopkins University School of Medicine), Kang Shen (Stanford University), and John White (University of Wisconsin).

This course was supported with funds provided by the National Institute of Child Health and Human Development.

PARTICIPANTS

Arous, B.J., M.S., Ecole Normale Superieure, Paris, France
Butcher, R., Ph.D., Harvard Medical School, Boston, Massachusetts
Gillan, V., Ph.D., University of Glasgow, United Kingdom
Guo, Z., B.S., Harvard University, Cambridge, Massachusetts
Hatin, I., Ph.D., Universite Paris Sud, Orsay, France
Hinas, A., M.S., Swedish University of Agricultural Sciences, Uppsala, Sweden
Jagadeeswaran, R., Ph.D., University of Chicago Medical Center, Illinois
Kikis, E., B.A., Northwestern University, Evanston, Illinois
Kirstein, J., M.S., Free University Berlin, Germany
Knoefler, D., M.S., University of Michigan, Ann Arbor
Li, X.-P., Ph.D., Rutgers, The State University of New Jersey, New Brunswick
Neveu, P., M.S., Ecole Normale Superieure, Paris, France
Rhode, C., M.S., Massachusetts Institute of Technology, Cambridge
Salinas, G., Ph.D., Universidad de la Republica, Uruguay
Salomon, M., B.S., University of Florida, Gainesville
Simonsen, K., M.S., University of Southern Denmark, Odense M, Denmark

SEMINARS

Ahmed, S., University of North Carolina, Chapel Hill: DNA repair.
de Bono, M., MRC-Laboratory of Molecular Biology, Cambridge, United Kingdom: Behavior.
Desai, A., University of California, La Jolla: Cell biology of the early embryo.
Dillin, A., The Salk Institute for Biological Studies, La Jolla, California: Aging.
Driscoll, M., Rutgers, The State University of New Jersey, Piscataway: Neurodegeneration.
Felix, M.-A., Institut Jacques Monod, CNRS, Paris, France: Evolution and vulva formation.
Fraser, A., The Wellcome Trust Sanger Institute, Hinxton, United Kingdom: RNAi.
Grant, B.,, Rutgers, The State University of New Jersey, Piscataway: Endocytosis.
Hodgkin, J., University of Oxford, United Kingdom: Pathogenesis and immunity.
Seydoux, G., Johns Hopkins University School of Medicine, Baltimore, Maryland: Germ cell fate.
Shen, K., Stanford University, California: Synapse formation.
White, J., University of Wisconsin, Madison: Microscopy.

X-Ray Methods in Structural Biology

October 15–30

INSTRUCTORS **W. Furey,** V.A. Medical Center/University of Pittsburgh, Pennsylvania
G. Gilliland, Centorcor R&D, Inc., Radnor, Pennsylvania
A. McPherson, University of California, Irvine
J. Pflugrath, Rigaku Americas Corporation, The Woodlands, Texas

ASSISTANT **J. Luo,** Centocor R&D, 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. Each student crystallized several proteins and determined at least one structure by several methods, in parallel with lectures on the theory and informal discussions behind the techniques.

Speakers in the course included Paul Adams, Zbigniew Dauter, Paul Emsley, Wayne Hendrickson, Xinhua Ji, Leemor Joshua-Tor, Morten Kjeldgaard, Gerard Kleywegt, Anastassis Perrakis, Randy Read,

David Richardson, Jane Richardson, Robert Sweet, Thomas Terwilliger, Dale Tronrud, David Waugh, and John Westbrook.

This course was supported with funds provided by the National Cancer Institute and the Howard Hughes Medical Institute.

PARTICIPANTS

Bick, M., B.S., The Rockefeller University, New York
Chan, S., Ph.D., NIEHS, Research Triangle Park, North Carolina
Forsgren, N., M.S., Umea University, Sweden
Kalathur, R., Ph.D., National Cancer Institute, Frederick, Maryland
Koharudin, L., Ph.D., University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania
Langelier, M.-F., Ph.D., Thomas Jefferson University, Philadelphia, Pennsylvania
Lee, J., B.A., Mayo Graduate School, Rochester, Minnesota
Magliery, T., Ph.D., The Ohio State University, Columbus
Magnusdottir, A., B.S., Karolinska Institutet, Stockholm, Sweden
Mehboob, S., Ph.D., University of Illinois, Chicago
Mohanty, S., Ph.D., Auburn University, Alabama
Noinaj, N., B.A., University of Kentucky College of Medicine, Lexington
Qi, Y., B.S., Harvard University, Cambridge, Massachusetts
Ren, X., Ph.D., National Institutes of Health, Bethesda, Maryland
Schaaf, G., Ph.D., University of North Carolina, Chapel Hill
Stuwe, T., B.S., EMBL Heidelberg, Germany

SEMINARS

Adams, P., Lawrence Berkeley Laboratory, Berkeley, California: Introduction to PHENIX (& CNS). Structure refinement.
Dauter, Z., Argonne National Laboratory, Illinois: Anomalous data collection.
Emsley, P., University of York, United Kingdom: Model-building tools in coot.
Furey, W., V.A. Medical Center/University of Pittsburgh, Pennsylvania: Patterson group therapy. Isomorphous replacement and anomalous scattering. Noncrystallographic symmetry averaging. MAD phasing, a classical approach. Solving flattening/pPhase combination. Solving structures with BnP.
Gilliland, G., Centocor, Inc., Radnor, Pennsylvania: Course overview: The structure determination of biological macromolecules. Crystallization strategies.
Hendrickson, W., Columbia University, New York: MAD phasing: Theory and practice.
Ji, X., National Institute of Standards & Technology, Rockville, Maryland: Structural basis for dsRNA processing RNase III: How Dicer dices. Structure presentation. DNA translocation in a replicative hexameric helicase.
Kjeldgaard, M., Aarhus University, Denmark: Electron density fitting from A to O.
Kleywegt, G., University of Uppsala, Sweden: Just because it's in Nature doesn't mean it's true... (macromolecular structure validation).
McPherson, A., University of California, Irvine: Crystallization of macromolecules I. Crystallization of macromolecules II. Symmetry, periodicity, unit cells, space groups, Miller planes, and lattices. Waves, vectors, and complex numbers. Fundamental diffraction relationships and Bragg's law. Diffraction patterns, reciprocal space, and Ewald's sphere. Fourier transforms and the electron density equation. Patterson techniques. Mechanisms of crystal growth. Heavy atoms and anomalous scatterers. Crystallization review.
Perrakis, A., Netherlands Cancer Institute, Amsterdam, The Netherlands: Automated model building and refinement with ARP/W/ARP.
Pflugrath, J., Rigaku Americas Corporation, The Woodlands, Texas: Data collection: Design and setup I. Data collection: Design and setup II. Cryocrystallography. Scaling and merging synchrotron data. Away from the edge: Sulfur SAD with chromium radiation.
Read, R., University of Cambridge, United Kingdom: Molecular replacement: From Pattersons to likelihood with a SAD commentary. Likelihood and experimental phasing.
Richardson, D. and Richardson, J., Duke University Medical Center, Durham, North Carolina: Detection and repair of model errors using MolProbity.
Sweet, R., Brookhaven National Laboratory, Upton, New York: Fundamental of crystallography. X-ray sources and optics.
Terwilliger, T., Los Alamos National Laboratory, Los Alamos, New Mexico: Automated structure solution and model-building.
Tronrud, D., University of Oregon, Eugene: Macromolecular refinement I. Macromolecular refinement II. Difference electron density maps. The structure of the FMO protein at 1.3 Å resolution.
Waugh, D., National Cancer Institute, Frederick, Maryland: Protein engineering for X-ray crystallographers.
Westbrook, J., Rutgers, The State University of New Jersey, Pisacataway: Automating PDB deposition.

Programming for Biologists

October 17–30

INSTRUCTORS **S. Lewis,** University of California, Berkeley
S. Prochnik, DOE–Joint Genome Institute/University of California, Berkeley
L. Stein, Cold Spring Harbor Laboratory
J. Tisdall, DuPont Experimental Station, Wilmington, Delaware

ASSISTANTS **J. Aerts,** Bioinformatics Group, Scotland, United Kingdom
J. Babayev, Pioneer Hi-Bred, Johnston, Iowa
J. Leipzig, DuPont Experimental Station, Wilmington, Delaware
T. Marques, University of Washington, Seattle
S. Robb, University of Utah, Salt Lake City
G. Thorisson, University of Leicester, United Kingdom

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Today the computer is an indispensable part of a research biologist’s toolkit. The success of the human and other organism genome projects has created terabytes of data on everything from genetic linkage mapping, to nucleotide sequences, to protein structures, stashed away in databases around the globe. Large-scale technologies such as DNA microarrays and high-throughput genotyping have transformed the nature of laboratory experimentation. Furthermore, even when biologists are not generating large data sets of their own, they will want to collect and analyze data from myriad sources in the pursuit of novel candidates or even entire research avenues.

A few years ago, it might have been sufficient to use Excel spreadsheets for managing laboratory data and canned Web interfaces for searching, but as the volume of data grows and the subtlety of

analysis increases, these techniques, even supplemented by some simple programming skills, have become inadequate. Modern biologists must be adept at juggling disparate data sets in order to pursue their research. Designed for students and researchers with some prior programming experience, this 2-week Advanced Bioinformatics program gave biologists the expanded bioinformatics skills necessary to construct computational systems that can exploit this increasingly complex information landscape, with an emphasis on fitting the wide range of existing analysis tools into extensible bioinformatics systems.

The course combined formal lectures with hands-on sessions in which students worked to solve a series of problem sets covering common scenarios in the acquisition, validation, integration, analysis, and visualization of biological data. For their final projects, students posed problems using their own data and worked with one another and the faculty to solve them. The prerequisites for the course were a basic knowledge of UNIX, procedural Perl programming, HTML document creation, and the database query language SQL. Lectures and problem sets covering this background material were available online, and students studied this material before starting the course.

Note that the primary focus of this course was to provide students with practical programming experience, rather than to present a detailed description of the algorithms used in computational biology. For the latter, we recommended the Computational and Comparative Genomics course.

Speakers in the 2007 course included Tyler Alioto, Emina Begovic, Scott Cain, George Hartzell, Matt Hibbs, Gabor Marth, Jason Stajich, Paul Thomas, and Mark I.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Beutler, A., Ph.D., Mt. Sinai School of Medicine, New York
Bharti, A., Ph.D., Rutgers, The State University of New Jersey, Piscataway
Burbano, H., M.Sc., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Carre, W., Ph.D., Roslin Institute, Roslin, United Kingdom
Clay, I., M.Sc., Cambridge University, Cambridge, United Kingdom
Ding, Y., Ph.D., The Jackson Laboratory, Bar Harbor, Maine
Fu, F., M.S., Iowa State University, Ames
Hall, I., Ph.D., Cold Spring Harbor Laboratory
Hancock, A., B.S., University of Chicago, Illinois
Haritunians, T., Ph.D., Cedars-Sinai Medical Center, Los Angeles, California
Kalatskaya, I., Ph.D., Universite de Montreal, Canada
Lashbrook, N., B.A., Stony Brook University, New York
Lindberg, J., M.S., Royal Institute of Technology, Stockholm, Sweden
Llaca, V., Ph.D., DuPont, Wilmington, Delaware
Maricic, T., M.Sc., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
Merkel, A., M.Sc., University of Canterbury, Christchurch, New Zealand
Mihindukulasuriya, K., Ph.D., Washington University, St. Louis, Missouri
Nowick, K., Ph.D., Lawrence Livermore National Laboratory, Livermore, California
Pelizzola, M., Ph.D., Yale University, New Haven, Connecticut
Roda, O., B.S., Institute for Systems Biology, Seattle, Washington
Tecle, I., Ph.D., Cornell University, Ithaca, New York
Tsukuda, T., Ph.D., University of New Mexico, Albuquerque
Yuhki, N., Ph.D., National Cancer Institute, Frederick, Maryland

SEMINARS

Alioto, T., University Pompeu Fabra, Barcelona, Spain: Gene prediction.
Begovic, E., University of California, Berkeley: Multiple sequence alignment + molecular evolution 1. Molecular evolution.
Cain, S., Cold Spring Harbor Laboratory: Gbrowse.
Hartzell, G., University of California, Berkeley: Database design. Perl DBI (Rose: DB). Perl and HTML. Perl and CGI. Good programming elective.
Hibbs, M., Princeton University, New Jersey: Microarrays.
Lewis, S., University of California Berkeley: Perl III.
Marth, G., Boston College, Chestnut Hill, Massachusetts: Sequence variation.
Prochnik, S., DOE–Joint Genome Institute/University of California, Berkeley: Perl VI. Perl review VII (writing OOP modules). Perl pipelines.
Stajich, J., University of California, Berkeley: BioPerl I. BioPerl II.
Stein, L., Cold Spring Harbor Laboratory: Perl I. Perl II. Perl V.
Thomas, P., SRI International, Menlo Park, California: Protein functional assignment.
Tisdall, J., DuPont Experimental Station, Wilmington, Delaware: Scientific computing elective.
Yandell, M., University of Utah, Salt Lake City: Blast.

Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging

October 18–31

INSTRUCTORS **V. Allan,** University of Manchester, United Kingdom
K. Hu, Indiana University, Bloomington
S. Huang, Northwestern University School of Medicine, Chicago, Illinois
J. Murray, University of Pennsylvania School of Medicine, Philadelphia
J. Waters, Harvard Medical School, Boston, Massachusetts

ASSISTANTS **C. Wang,** Northwestern University Medical School, Chicago, Illinois
M. Wozniak, University of Manchester, United Kingdom

This course focused on specialized techniques in microscopy, in situ hybridization, immunocytochemistry, and live cell imaging related to localizing DNA, RNA, and proteins in fixed cells, as well as protein and RNA dynamics in living cells. It emphasized the use of the latest equipment and techniques in fluorescence microscopy, including confocal laser-scanning microscopy, deconvolution methods, 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. It was also 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, and the use of GFP variants to study protein expression, localization, and dynamics. In each method, several experimental protocols were presented allowing the students to assess the relative merits of each and to relate them to their own research. Students were encouraged to bring their own nucleic acid, protein, or antibody probes to the course, which were used in addition to those provided by the instructors. The laboratory exercises were supplemented with lectures given by invited distinguished scientists, who present up-to-the-minute reports on current methods and research using the techniques being presented.

Speakers in the course included Richard Day, Joseph Gall, Rudolf Oldenbourg, Thomas Ried, Greenfield Sluder, and David Spector.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Bonilla, D., B.Sc., Texas A&M Health Science Center, College Station
Chen, M., Ph.D., The Salk Institute, La Jolla, California
Evans, A., B.S., University of Texas, Austin, Port Aransas
Fulcher, J., B.S., University of California, Los Angeles
Galifianakis, N., Ph.D., New York University School of Medicine, New York
Gumy, L., Ph.D., University of Cambridge, United Kingdom
Henriksson, S., M.S., Uppsala University, Sweden
Hollien, J., Ph.D., University of California, San Francisco
Kumanova, M., M.D., University of Pennsylvania, Philadelphia
Nedbal, J., M.Sc., King's College London, United Kingdom
Proenca, C., B.A., Weill Cornell Medical College, New York
Scahill, C., B.A., Cambridge University, United Kingdom
Soong, B.-W., Ph.D., National Yang-Ming University Faculty of Medicine, Taipei, Taiwan
Weech, M., Ph.D., University of North Carolina, Chapel Hill
Williams, J., B.S., Cold Spring Harbor Laboratory
Ye, X., M.S., University of California, Irvine

SEMINARS

Allan, V., University of Manchester, United Kingdom: Immunocytochemistry.
Day, R., University of Virginia, Charlottesville: Seeing colors: Applications and limitations of the fluorescent proteins.
Gall, J., Carnegie Institution, Baltimore, Maryland: The development of in situ hybridization.
Greenfield, S., University of Massachusetts Medical School, Worcester: You can observe a lot just by watching: The charm of live cell analysis.
Hu, K., Indiana University, Bloomington and Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Basic introduction to light and fluorescence microscopy.
Huang, S., Northwestern University School of Medicine, Chicago, Illinois: Introduction to FISH.
Murray, J., University of Pennsylvania School of Medicine, Philadelphia: Other microscopy methods for live cell imaging. Confocal microscopy and other thick-specimen techniques.
Oldenbourg, R., Marine Biological Laboratory, Woods Hole, Massachusetts: Live cell imaging with polarization microscopy.
Ried, T., National Cancer Institute/NIH, Bethesda, Maryland: Mechanisms and consequences of chromosomal aberrations in cancer cells.
Spector, D., Cold Spring Harbor Laboratory: Localization of gene expression by FISH and in living cells.
Waters, J., Harvard Medical School, Boston, Massachusetts: Digital detectors and digital imaging fundamentals.

Revolutionary Sequencing Technologies and Applications

November 6–17

INSTRUCTORS
G. Hannon, Cold Spring Harbor Laboratory
E. Mardis, Washington University School of Medicine, St. Louis, Missouri
G. Marth, Boston College, Chestnut Hill, Massachusetts
W. McCombie, Cold Spring Harbor Laboratory
J. McPherson, Baylor College of Medicine, Houston, Texas
M. Zody, Broad Institute, Cambridge, Massachusetts

ASSISTANTS
A. Briggs, Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany
J. Glasscock, Washington University School of Medicine, St. Louis, Missouri
M. Hickenbotham, Washington University School of Medicine, St. Louis, Missouri
V. Magrini, Washington University School of Medicine, St. Louis, Missouri
A. Quinlan, Boston College, Allston, Massachusetts
D. Stewart, Boston College, Chestnut Hill, Massachusetts

During the last decade, large-scale DNA sequencing has markedly impacted the practice of modern biology and is beginning to affect the practice of medicine. With the recent introduction of several revolutionary sequencing technologies, costs and timelines have been reduced by orders of magnitude, facilitating investigators to conceptualize and perform sequencing-based projects that heretofore were prohibitive. Furthermore, the application of these technologies to answer questions previously not experimentally approachable is broadening their impact and application.

This intensive 12-day course explored applications of next-generation sequencing technologies, with a focus on commercially available methods. Students were instructed in the detailed operation of several revolutionary sequencing platforms, including sample preparation procedures, general data handling

through pipelines, and in-depth data analysis. A diverse range of biological questions were explored, including DNA resequencing of human genomic regions (using cancer samples as a test case), de novo DNA sequencing of bacterial genomes, and the use of these technologies in studying small RNAs, among others. Guest lecturers highlighted their own applications of these revolutionary technologies. We encouraged applicants from a diversity of scientific backgrounds including molecular evolution, development, neuroscience, cancer, plant biology and microbiology.

This course was sponsored equally by Applied Biosystems, Illumina, and 454 Life Sciences.

PARTICIPANTS

Caudy, A., Ph.D., Princeton University, New Jersey
Duan, S., Ph.D., University of Massachusetts Medical School, Worcester
Farrell, A., B.A., GeneDx, Gaithersburgh, Maryland
Figueiredo, L., M.Sc., Federal University of Minas Gerais, Belo Horizonte, Brazil
Hall, I., Ph.D., Cold Spring Harbor Laboratory
Hlavata, I., M.Sc., National Institute of Public Health, Czech Republic
Hoberman, R., B.A., McGill University, Montreal, Quebec City, Canada
Liljedahl, U., Ph.D., Uppsala University, Sweden
Lofts, L., M.Sc., USAMRIID, Frederick, Maryland
Peris, M., Ph.D., Applied Biosystems, Foster City, California
Pradervand, S., Ph.D., University of Lausanne, Switzerland
Sisneros, N., B.S., University of Arizona, Tucson
Stromberg, M., Ph.D., Boston College, Chestnut Hill, Massachusetts
Yeo, G., Ph.D., Salk Institute, La Jolla, California

SEMINARS

Briggs, A., Max-Planck Institute for Evolutionary Anthropology, Leipzig, Germany: Massively parallel sequencing of ancient DNA.
Gingeras, T., Affymetrix, Santa Clara, California: Genome-wide transcription maps reveal an interleaved organization of functional elements and likely regulatory strategies.
Hannon, G., Cold Spring Harbor Laboratory: miRNAs and biology.
Hercus, C., Synamatix, Kuala Lumpur, Malaysia: Synamatix-based search and analysis approaches using massively parallel sequence data.
Mikkelsen, T., Broad Institute, Cambridge, Massachusetts: Genome-wide maps of chromatin state in pluripotent and lineage-committed cells.

Phage Display of Proteins and Peptides

November 6–17

INSTRUCTORS **C. Barbas,** The Scripps Research Institute, La Jolla, California
D. Siegel, University of Pennsylvania School of Medicine, Philadelphia
G. Silverman, University of California, La Jolla

ASSISTANTS **C. Gronwall,** Royal Institute of Technology, Stockholm, 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 the library. Students learned the theoretical and practical aspects of constructing combinatorial libraries from immune and nonimmune sources, as well as the construction of synthetic antibody libraries. Antibodies were selected from the library by panning. Production, purification, and characterization of antibody fragments expressed in *E. coli* were also covered. Peptide phage display was used to identify a monoclonal antibody's epitope.

The lecture series, presented by a number of invited speakers, emphasized PCR of immunoglobulin genes, the biology of filamentous phage and the utility of surface expression libraries, expression of antibodies in *E. coli* and mammalian cells, antibody structure and function, catalytic antibodies, directed protein evolution, 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 were also explored.

Seminar speakers in the course included Carlos Barbas, Germaine Fuh, Caroline Gronwall, Robert Charles Ladner, Christopher Noren, Sachdev Sidhu, Don Siegel, Gregg Silverman, Robyn Stanfield, and K. Dane Wittrup.

This course was supported with funds provided by the Howard Hughes Medical Institute.

PARTICIPANTS

Brenner-Morton, S., M.S., HHMI/Columbia University, New York
Chen, Y., Ph.D., Sanofi Aventis, Bridgewater, New Jersey
Cottier, V., Ph.D., ESBATech AG, Zurich-Schlieren, Switzerland
Cullen, P., Ph.D., Monash University, Victoria, Australia
Gilchuk, P., M.S., Institute of Molecular Biology and Genetics, Kyiv, Ukraine
Hopkins, J., B.Sc., University of Queensland, St. Lucia, Queensland, Australia
Karhu, N., M.Sc., Next Biomedical Technologies, Helsinki, Finland
Leong, S., Ph.D., University of Wisconsin, Madison
Molano, I., B.S., Medical University of South Carolina, Charleston
Piterina, A., M.S., University of Limerick, Republic of Ireland
Soerensen, M., M.S., University of Aarhus, Denmark
Tassev, D., B.S., Memorial Sloan-Kettering Cancer Center, New York
Velanac, V., Dipl., Max-Planck Institute for Experimental Medicine, Goettingen, Germany
Walper, S., B.S., The University of Southern Mississippi, Hattiesburg
Weiss, Y., M.S., Tel Aviv University, TIsrael

SEMINARS

Barbas, C., The Scripps Research Institute, La Jolla, California: Software and hardware for genomes: Polydactyl zinc finger proteins and the control of endogenous genes.
Fuh, G., Genetech, Inc., San Francisco, California: Use of phage display in development of therapeutic antibodies.
Ladner, R.C., Dyax Corporation, Cambridge, Massachusetts: Phage display of peptides and Kunitz domains.
Noren, C., New England BioLabs, Beverly, Massachusetts: Phage peptide libraries: The Ph.D. for peptides.
Sidhu, S., Genetech, Inc., San Francisco, California: Antibody phage display and chemical diversity in antigen recognition.
Siegel, D., University of Pennsylvania Medical Center, Philadelphia: Cell surface selection of combinatorial antibody libraries.
Silverman, G., University of California, San Diego, La Jolla: Repertoire cloning of SLE autoantibodies.
Stanfield, R., The Scripps Research Institute, La Jolla, California: Stuctural molecular biology of antibodies.
Wittrup, K.D., Massachusetts Institute of Technology, Cambridge: Yeast display libraries.

Proteomics

November 6–19

INSTRUCTORS **P. Andrews,** University of Michigan Medical School, Ann Arbor
J. La Baer, Harvard Institute of Proteomics, Cambridge, Massachusetts
A. Link, Vanderbilt University School of Medicine, Nashville, Tennessee

ASSISTANTS **R. Bish,** Cold Spring Harbor Laboratory
A. Farley, Vanderbilt University, Nashville, Tennessee
E. Hainsworth, Harvard Medical School, Cambridge, Massachusetts
J. Jennings, Vanderbilt University, Nashville, Tennessee
E. Simon, University of Michigan, Ann Arbor
S. Srivastava, Harvard University, Cambridge, Massachusetts
S. Volk, University of Michigan, Blissfield

This intensive laboratory and lecture course focused on two major themes in proteomics: protein profiling and functional proteomics. In the profiling section of the course, students learned about cutting-edge protein separation methods, including hands-on experience with 2D gel electrophoresis, multidimensional liquid chromatography, and affinity purification of protein complexes. The course covered both MALDI and ESI high-sensitivity mass spectrometry, including peptide mass mapping and tandem mass spectrometry, quantification, and phosphoproteomics.

Students learned to use several informatics tools available for analyzing the data. In the functional proteomics section of the course, students learned about recombinational cloning, high-throughput protein isolation, and protein microarrays. They used robots to execute high-throughput methods including

expression, purification, and characterization of proteins. They also printed and analyzed their own self-assembling protein microarrays, which were used for protein–protein interaction studies. 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.

Speakers in the course included Ron Beavis, Claudio Calonder, Brian Chait, Pierre Chaurand, Karl Clauser, Paul Huang, Darryl Pappin, Michael Synder, David Tabb, Paul Tempst, and Ileana Cristea.

This course was supported with funds provided by the National Cancer Institute.

PARTICIPANTS

Agwu, E., M.M.L.S., Kampala International University, Bushenyi, Uganda
Altug, H., B.S., Boston University, Massachusetts
Barnes, A., Ph.D., University of North Carolina, Chapel Hill
Dam, S., M.Sc., University of Aarhus, Denmark
Dinglasan, R.D., Ph.D., Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland
Fernandez, E., Ph.D., Wellcome Trust Sanger Institute, Cambridge, United Kingdom
Hand, R., B.S., University of North Carolina, Chapel Hill
Kim, J., Ph.D., Washington University School of Medicine, St. Louis, Missouri
McKenney, P., B.S., New York University, New York
Novak, S., Ph.D., Agriculture Research Division, Edmonton, Canada
Nerelius, C., M.S, Swedish University of Agricultural Sciences, Uppsala
Oakley, F., B.S., University of Iowa, Iowa City
Ruggiero, A., Ph.D., Vanderbilt University, Nashville, Tennessee
Singh, M., M.Sc., International Centre for Genetic Engineering and Biotechnology, New Delhi, India

SEMINARS

Beavis, R., University of British Columbia, Vancouver, B.C., Canada: Proteomics bioinformatics.
Chait, B., The Rockefeller University, New York: Proteomic analysis of protein complexes.
Chaurand, P., Vanderbilt University School of Medicine, Nashville, Tennessee: Tissue imaging and profiling by mass spectrometry.
Clauser, K., Broad Institute/Massachusetts Institute of Technology: De novo interpretation of tandem mass spectra.
Colonder, C., Zeptosens Bioanalytical Solutions, Switzerland: Reverse protein arrays.
Cristea, I., The Rockefeller University, New York: Analysis of protein–protein interactions via immunoaffinity purifications.
Huang, P., Massachusetts Institute of Technology, Charlestown: Phosphoproteomics.
Pappin, D., Applied Biosystems, Framingham, Massachusetts: Quantative proteomics.
Synder, M., Yale University, New Haven, Connecticut: Application of protein arrays.
Tabb, D., Vanderbilt University, Nashville, Tennessee: Mass spectrometry data analysis.
Tempts, P., Memorial Sloan-Kettering Cancer Center, New York: Serum biomarker.

Computational and Comparative Genomics

November 7–13

INSTRUCTORS **W. Pearson,** University of Virginia, Charlottesville
R. Smith, GlaxoSmithKline, King of Prussia, Pennsylvania

ASSISTANT **D. Triant,** Louisana State University, Baton Rouge

This course presented a comprehensive overview of the theory and practice of computational methods for gene identification and characterization from DNA sequence data. The course focused on approaches for extracting the maximum amount of information from protein and DNA sequence similarity through sequence database searches, statistical analysis, and multiple sequence alignment, whole-genome analysis, and comparative genomics. Additional topics included identifying signals in unaligned sequences and integration of genetic and sequence information in biological databases. The course combined lectures with hands-on exercises; students were encouraged to pose challenging sequence analysis problems using their own data.

The course made extensive use of local WWW pages to present problem sets and the computing tools to solve them. Students used Windows and Mac workstations attached to a UNIX server; participants were comfortable using the Unix operating system and a Unix text editor. It was designed for biologists seeking advanced training in biological sequence 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.

The primary focus of this course was the theory and practice of algorithms used in computational biology, with the goal of using current methods more effectively and developing new algorithms. Students more interested in the practical aspects of advanced software development were encouraged to apply to the course on Programming for Biology.

Speakers in the course included Stephen Altschul, Peter D'Eustachio, Eric Green, Ross Hardison, Eric Sayers, Cynthia Spudich, and James Taylor.

This course was supported by the National Human Genome Research Institute.

PARTICIPANTS

Abraham, D., Ph.D., University of Virginia, Charlottesville
Budroni, S., M.S., Perugia University, Perugia, Italy
Bush, E., Ph.D., Harvey Mudd College, Claremont, California
Cheng, M.L., B.S., Celera, Rockville, Maryland
Kahsay, R., Ph.D., Dupont CR&D, Wilmington, Delaware
Lessick, R., Ph.D., Johns Hopkins University, Baltimore, Maryland
Levenstien, M., Ph.D., Memorial Sloan-Kettering Cancer Center, New York
Noli, A., M.S., Stowers Institute for Medical Research, Kansas City, Missouri
Patel, D., M.Sc., University of Nottingham, Loughborough, United Kingdom
Plata, G., B.S., International Center for Tropical Agriculture, Cali, Columbia
Ryder, E., Ph.D., Worcester Polytechnic Institute, Pennsylvania
Sales, G., M.Sc., Universita degli Studi di Torino, Torino, Italy
Samollow, P., Ph.D., Texas A&M University, College Station
Sethuraman, A., Ph.D., Stanford Human Genome Center, Palo Alto, California

SEMINARS

Altschul, S., National Library of Medicine, Bethesda, Maryland: Statistics of sequence similarity scores. Iterated protein database searches with PSI-BLAST.
D'Eustachio, P., New York University School of Medicine, New York: Representing biological processes: Reactome and other pathway databases.
Green, E., Genome Technology Branch, Bethesda, Maryland: Inter- and intraspecies sequence comparisons: Deducing genome function in health and disease.
Hardison, R., Pennsylvania State University, University Park, and Taylor, J., New York University, New York: Comparative genomics I: Tools for comparative genomics. Genome computation and gene regulation.
Pearson, W., University of Virginia, Charlottesville: Alignment algorithms: Large-scale alignment. Protein evolution and sequence similarity searching. Algorithms for biological sequence comparison. Hidden Markov models and protein profiles. Identifying consensus sites.
Sayers, E., National Library of Medicine, Bethesda, Maryland: NCBI resources for bioinformatics and computational biology. NCBI genome resources.
Smith, R., GlaxoSmithKline, King of Prussia, Pennsylvania: Approaches to multiple sequence alignment.
Spudich, G., EMBL–European Bioinformatics Institute, Hinxton, United Kingdom: The ENSEMBL database of genomes I. ENSEMBL/Biomart II.

The Genome Access Course

April 24–25, August 28–29, November 28–29

TRAINERS **G. Howell,** The Jackson Laboratory
B. King, The Jackson Laboratory
L. Reinholdt, The Jackson Laboratory
R. Sachidanandam, Cold Spring Harbor Laboratory

The Genome Access Course is an intensive 2-day introduction to bioinformatics that was held multiple times in 2003 and trained almost 110 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: 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 newly opened 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 24–25

Afonina, I., Nanogen Inc., Bothell, Washington
Al-Assah, R., Abu Dhabi University, United Arab Emirates
Burdick, K., Zucker Hillside Hospital-NSLIJHS, Glen Oaks, New York
De Oliveira, A., Federal University of Pelotas, Brazil
DeRosse, P., Zucker Hillside Hospital, Glen Oaks, New York
DeWille, J., Ohio State University, Columbus
Deys, K., USDA-ARS, Geneva, New York
Gillmor, S., University of Pennsylvania, Philadelphia
Goldberg, A., Oakland University, Rochester, Michigan
Gomos-Klein, J., City University of New York, Hunter College, New York
Gutierrez-Nava, M. de la Luz, DuPont, Wilmington, Delaware
He, M., Cold Spring Harbor Laboratory
Inlow, J., Indiana State University, Terra Haute
Iovine, N., New York University School of Medicine, New York
Lahiji, A., City University of New York, Hunter College, New York
Lencz, T., The Zucker Hillside Hospital, Glen Oaks, New York
Lin, P.I., Cold Spring Harbor Laboratory
Liu, G., University of Iowa, Iowa City
Mills, A., Cold Spring Harbor Laboratory
Min, M., Correlagen Diagnostics, Inc., Waltham, Massachusetts
Newton, S., University of Iowa, Iowa City
Paul, A., Cold Spring Harbor Laboratory
Rakoff-Nahoum, S., Yale University School of Medicine, New Haven, Connecticut
Rivera, R., University of Pennsylvania, Philadelphia
Rogoz, A., City University of New York, Hunter College, New York
Rojas, J., Regeneron Pharmaceuticals, Tarrytown, New York
Shumay, E., Brookhaven National Laboratory, Upton, New York
Stein, P., University of Pennsylvania, Philadelphia
Sun, A.-Q., Mount Sinai School Medicine, New York
Tom, H.-I, The Johns Hopkins University, Baltimore, Maryland
Vermeulen, N., Nanogen Corporation, Bothell, Washington
Wang, W., Memorial Sloan-Kettering Cancer Center, New York
Zieve, G., Stony Brook University, New York

August 28–29

Baker, B., Yale University, New Haven, Connecticut
Buchholtz, E., Wellesley College, Massachusetts
Carr, E., Raritan Valley Community College, Somerville, New Jersey
Cepko, C., Harvard Medical School, Boston, Massachusetts
Chen, T.-H,, Boston University, Cambridge, Massachusetts
Johnson, S., Cardinal Health, Milford Center, Ohio
Lefterova, M., University of Pennsylvania, Philadelphia
Li, J., Wayne State University, Detroit, Michigan
Miller, R., The Johns Hopkins University, Baltimore, Maryland
Pelc, T., Midwest Research Institute, Rockville, Maryland
Perumbeti, A., Cincinnati Children's Hospital Medical Center, Ohio
Ramachandran, B., Amgen Inc., South San Francisco, California
Rule, A., Mayo Clinic, Rochester, Minnesota
Stepien, C., Wellesley College, Massachusetts
Weiss, S., Brigham and Women's Hospital, Boston, Massachusetts
Yang, G., Mount Sinai School of Medicine, New York
Yi, R., The Rockefeller University, New York

November 28–29

Alcantara, S., University of Texas Southwestern Medical Center, Dallas
Andrews, Z., Yale University, New Haven, Connecticut
Broder-Fingert, S., Massachusetts General Hospital, Boston
Cidado, J., Novartis (NIBRI), Cambridge, Massachusetts
Enzmann, B., University of California, Los Angeles
Felice, K., University of Connecticut Health Center, Farmington
Golovan, S., University of Guelph, Canada
Jesionowski, A., University at Buffalo, New York
Kapheim, K., University of California, Los Angeles
Kebede, H., Texas Tech University, Lubbock
Koh, I.S., HanYang University, College of Medicine, Seoul, South Korea
Kopplin, L., Case Western Reserve University, Cleveland, Ohio
Li, Z., Columbia University, New York
Lin, F., Novartis Institutes of BioMedical Resarch, Cambridge, Massachusetts
Litonjua, A., Brigham and Women's Hospital, Boston, Massachusetts
Marti-Subirana, A., Phoenix College, Arizona
McGrath, J.M., USDA-ARS, East Lansing, Michigan
Moschenross, D., University of Connecticut, Farmington
Muehlschlegel, J., Brigham and Women's Hospital, Boston, Massachusetts
Pal, D., Columbia University, New York
Reid Lombardo, K., Mayo Clinic, Rochester, Minnesota
Samten, B., University of Texas Health Center, Tyler
Sotolongo, B., University of Miami School of Medicine, Florida
Srivastava, D., Boston Biomedical Research Institute, Watertown, Massachusetts
Strauss, S., Oregon State University, Corvallis
Triche, E., Yale University School of Medicine, New Haven, Connecticut
Zheng, J., Carolinas HealthCare System, Charlotte, North Carolina

Cold Spring Harbor Laboratory acknowledges the generosity of the following companies who loaned equipment and reagents to the various courses:

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Torrey Pines Scientific
Universal Imaging Corporation
VDS Vosskuhler GmbH
Vector Laboratories
Warner Instruments
Waters Corp
Zeitz-Instrumente Vertriebs GmbH

SEMINARS

INVITED SPEAKER PROGRAM

Each year, Cold Spring Harbor Laboratory invites speakers from outside the institution to present their findings. These weekly seminars keep the CSHL staff current on the latest scientific developments and broaden their perspectives. Graduate students and postdoctoral fellows meet with the seminar speakers for lunch immediately after the seminar, allowing an opportunity for the exchange of ideas in an informal setting.

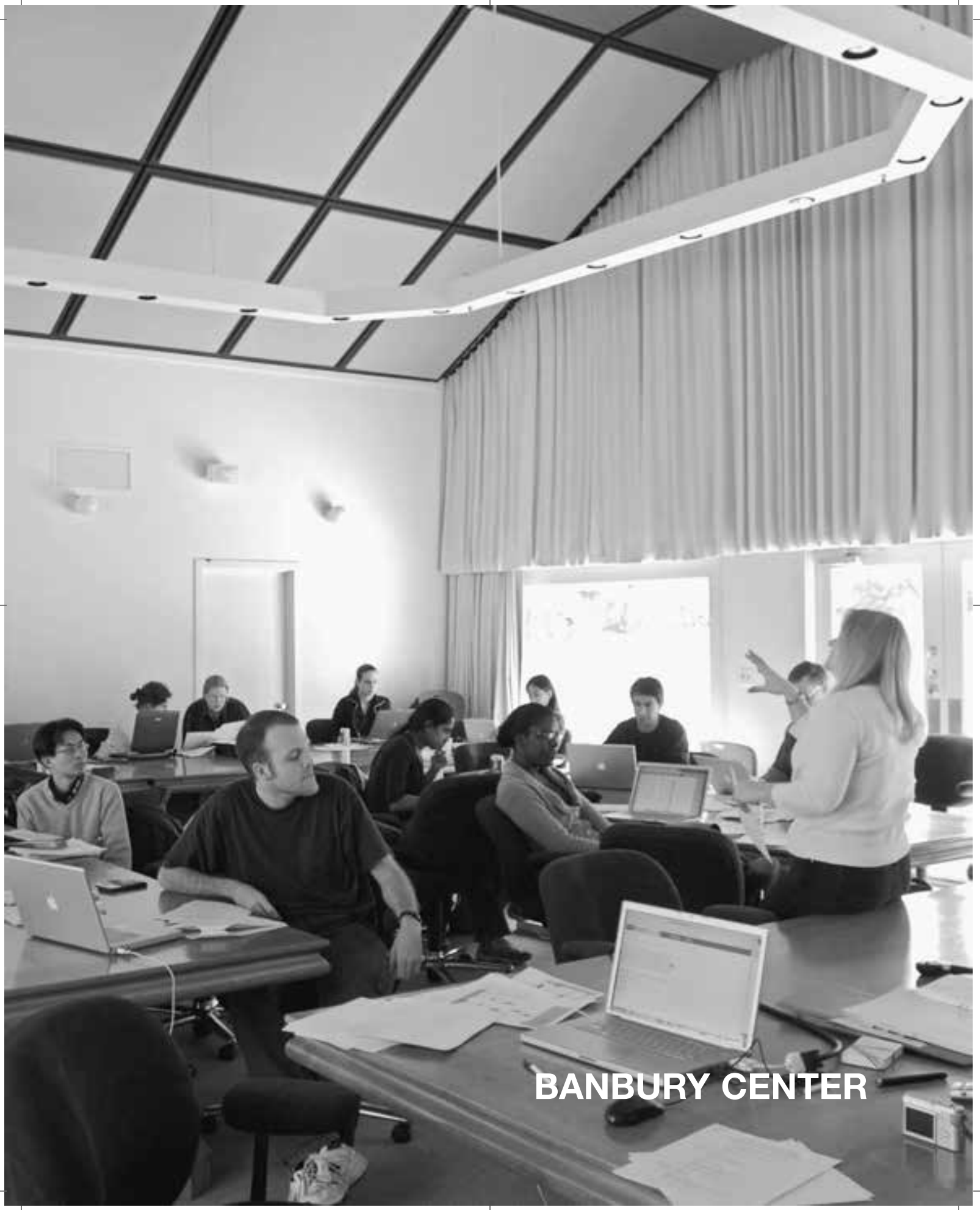
	Title	Host
January		
Dr. Mary Beth Hatten, The Rockefeller University	New direction in CNS migration	Linda Van Aelst
Dr. Ian Macara, University of Virginia	Parsing the polarity code: Polarization in epithelia and neurons	Linda Van Aelst
Dr. Christopher Lima, Memorial Sloan-Kettering Cancer Center	Posttranscriptional protein modification by the ubiquitin-like modifier SUMO	Bill Tansey
February		
Dr. Joan Massagué, Memorial Sloan-Kettering Cancer Center	Identifying metastasis genes and functions	Vivek Mittal
Dr. Todd Golub, Broad Institute of Harvard and Massachusetts Institute of Technology	Signatures for cancer discovery	Scott Powers
Dr. Arturo Alvarez-Buylla, University of California, San Francisco	Mosaic organization of adult neural stem cell niche	Grisha Enikolopov
Dr. Christoph Klein, Clinical Center of the University of Regensburg, Germany	From minimal systemic cancer to metastasis: Lessons from the direct analysis of single disseminated cancer cells	Yuri Lazebnik
March		
Dr. Brenton Graveley, University of Connecticut Health Center	Alternative splicing of Dscam: The making of 38,000 isoforms	Adrian Krainer
Dr. Max Wicha, University of Michigan	Self-renewal pathways in normal and malignant human mammary stem cells	Senthil Muthuswamy
Dr. Michael Greenberg, Children's Hospital of Boston, Harvard Medical School	Signaling networks that regulate synapse development and cognitive function	Linda Van Aelst
Dr. Susumu Tonegawa, Massachusetts Institute of Technology	Molecular, cellular, and circuit mechanisms for hippocampal memory	Josh Huang
Dr. Michael Dickinson, California Institute of Technology	How flies fly	Partha Mitra
April		
Dr. Liz Blackburn, University of California, San Francisco	Responses of cells and organisms to perturbing telomerase	Fabiola Rivas (Hannon Lab)
September		
Dr. Charles Sherr, St. Jude Children's Research Hospital	ARF tumor suppressor	Scott Lowe
Dr. Richard Myers, Stanford University	Global genomic analysis of transcription and inherited traits in humans	Adrian Krainer
October		
Dr. Oliver Hobert, Columbia University Medical Center	Regulatory logic of neuronal diversity in *C. elegans*	Josh Huang
Dr. James Carrington, Oregon State University	Specialized small RNA pathways in *Arabidopsis*	Marja Timmermans
Dr. Carol Greider, Johns Hopkins School of Medicine	Telomerase and the consequences of telomere dysfunction	Scott Powers

November		
Dr. Douglas Wallace, University of California, Irvine School of Medicine	A mitochondrial paradigm for metabolic and degenerative diseases, aging, and cancer	Terri Grodzicker
Dr. Matthew Scott, Stanford University School of Medicine	Communicating with hedgehogs	Josh Dubnau
December		
Dr. Antoine Van Oijen, Harvard Medical School	Under the hood of the peplisome: A single-molecule view of DNA replication	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	
Anindya Bagchi (Mills Lab)	Of mice and men: Road to the human *1p36* tumor suppressor
Ira Hall	The dynamic behavior of DNA copy-number variation in inbred mice
Marissa Nolan (Muthuswamy Lab)	Polarity proteins multitask: Par6 induces proliferation in breast epithelial cells
February	
Tom Gingeras	Genome-wide transcription maps reveal interleaved organization, new classes of RNAs, and possible function for pervasive transcription
Miguel Zaratiegui (Martienssen Lab)	Transcriptional landscape of a centromere
March	
Rita Gandhi (Hirano Lab)	Separating sisters (chromatids): When and where?
Tony Zador	Auditory cortex: From synapses to behavior
DingCheng Gao (Mittal Lab)	Targeting endothelial progenitor cells in tumor metastasis
Partha Mitra	The origin of EEG and LFP signals and a correction to the Hodgkin–Huxley equations for action potential propagation
Fabio Nogueira (Timmermans Lab)	Big roles for small RNAs in organ polarity
April	
Senthil Muthuswamy	Cell polarity: Defining a new regulatory mechanism for cancer progression
October	
Gonzalo Otazu (Zador Lab)	Task-dependent suppression of sound responses in auditory cortex
Alexei Koulakov	Activity and chemoaffinity in the development of neural maps
Chaolin Zhang (Zhang Lab)	Genome evolution and signals involved in fidelity and regulation of pre-mRNA splicing
November	
Patrick Paddison	The mammalian Swi/Snf complex is required for lineage commitment and specification in embryonic stem cells
Lin He (Hannon Lab)	microRNAs in the oncogenic and tumor suppressor network
Bruce Stillman	Control of the chromosome cycle in human cells by the origin recognition complex
December	
David Mu (Powers Lab)	Novel lung oncogenes and clinical implications
Scott Lowe	Dissecting tumor suppressor gene networks in vivo
Zhenyu Xuan (Zhang Lab)	Bioinformatics study in next generation sequencing



BANBURY CENTER

BANBURY CENTER
EXECUTIVE DIRECTOR'S REPORT

The year 2007 marked my 20th year as director of the Banbury Center. The Center continues to be a thrilling place to work, providing opportunities to help develop and promote new and important fields of research. The year proved to be one of the busiest ever in the 30 years since the first meeting was held in 1977. There were 25 scientific meetings, and the Center was used for nine courses. As usual, local organizations, including the Lloyd Harbor Conservation Board and the Cold Spring Harbor School District, made use of the calm of the Center for discussion meetings. Altogether, 36 events were held at Banbury in 2007, and these were attended by 672 participants whose demographics remain very similar from year to year. The proportion of participants from the United States was 77%, drawn from no fewer than 40 states, a record number. As usual, New York, California, Massachusetts, and Maryland provided most of these participants (46%). Foreign participants came from 23 countries, and attendance by scientists from companies was higher than in previous years, comprising 10% of the total.

There were two most interesting and important meetings dealing with social and policy issues relating to the uses of scientific knowledge. *Protecting Public Trust in Immunization*, funded by the Albert B. Sabin Vaccine Institute, has the potential to be one of the most important meetings to be held at Banbury. Vaccination against infectious agents is, unquestionably, the greatest contribution that science has made to improving our health, most especially that of children. Diseases such as polio are virtually forgotten in most countries; smallpox, the great scourge, has been eliminated; and a vaccine against human papillomaviruses is likely to reduce greatly the incidence of cervical cancer. But the absence of infections such as measles, mumps, and rubella has led to a perception that these, too, have disappeared, and the vaccination rates of the MMR (measles, mumps, rubella) vaccine have fallen. This has been accelerated by fears that the MMR vaccine, and/or the mercury once used as a preservative,

Banbury Lane

causes autism. Every study has shown that these fears are unfounded, but the perception remains and, as a consequence of the falling use of the MMR vaccine, these diseases are reappearing and killing children. Participants in this meeting discussed what can be done in general to persuade the public of the need for vaccination and to reestablish the public's trust in vaccines. A very distinguished group of participants included representatives of parent advocate organizations, the World Health Organization, the Department of Health and Human Services, Congress, and academic institutions. We were particularly pleased that Louis W. Sullivan, Secretary of Health and Human Services in the administration of George H.W. Bush, attended.

The second science and society discussion meeting also dealt with the vexed issue of how scientific knowledge is used and misused, particularly in the political decision-making process. To what extent should political influences determine what research is done and how scientific data are used? Contemporary examples include the U.S. government's attitudes toward human embryonic stem cell research and global warming. What is needed is a rational approach to decision-making, based on the best evidence available. *Retreat from Reason* reviewed these issues and explored how rationality might be reintroduced into public discourse. The participants were particularly eclectic, drawn from the worlds of science, politics, and the media. They included, for example, Michael Crichton (*Jurassic Park*), Chris Mooney (*Seed Magazine*), Lord Taverne (Sense about Science), and Lee Silver (Princeton University). It was a fascinating occasion.

The Alfred P. Sloan Foundation is noted for the support it gives to fledgling areas of research. The Foundation provides funds at a critical period when such fields may not yet have developed a community and have not produced a body of work sufficient to establish themselves for federal funding. Four years ago, in 2003, the Sloan Foundation supported two workshops at Banbury to discuss molecular "bar codes." These are based on the sequence of a mitochondrial gene, *COXI*, which is identical among individuals of a species but differs sufficiently among species so that it can be used to identify (bar-code) species. The Foundation's initiative has been tremendously successful. There is now a Consortium for the Barcode of Life, with 150-member organizations worldwide, coordinating and standardizing bar-code efforts. It is expected that a half-million species will be bar-coded during the next 5 years. The Sloan Foundation funded a follow-up discussion workshop at Banbury in 2007. Participants discussed both the effectiveness and limitations of bar coding, and it was agreed that bar coding is fulfilling its promise. Indeed, it is progressing so well that a major goal of the meeting was to explore how bar coding can be exploited beyond its present use in taxonomy. There were also important discussions of how to analyze and integrate the large amount of data being produced; effective visualization tools are essential for presenting these data in an interpretable fashion to researchers.

There were two particularly interesting meetings in neuroscience. The Swartz Foundation has supported meetings at Banbury since 1999, and the topics are always fascinating. *New Frontiers in Studies of Nonconscious Processing* was no exception. "Nonconscious processing" seems like a paradox—How can our brains process information without us being aware of it? And if it happens, how can we be aware that we are not aware of it happening?! A little reflection shows that a great deal of our brain activity must be devoted to nonconscious processes. What makes the topic intriguing is its implications for the classic and ever-lasting issue of free will. Experiments have indicated that we reach a decision about how to act before we become aware of the decision. So, our conscious mind, which we generally believe to be "in control" of such decisions, is not; the decision is made and then our conscious mind acts as though it reached the decision. The broad range of the topic was evident in the disciplines represented: social psychologists, cognitive psychologists, neural physiologists, and philosophers.

The second neuroscience meeting that was even broader in its coverage was *Interdisciplinary Memory Symposium in Neurosciences and the Humanities*. The primary focus of the meeting was on memory, the thesis being that, just as neuroscientists explore the physical workings of the brain with the tools of electrophysiology and molecular biology, so writers and artists explore and record the mental experiences of human beings. One thinks immediately of Proust's *À la recherche du temps perdu*. The participants explored the extent to which the insights of those in the humanities might guide neuroscientists in developing and evaluating their models of human memory. We moved from the genetics and

Back of Banbury Center; meeting coffee break

molecular biology of memory in fruit flies, through higher-level processes in more complex brains, to the role memory plays in art, literature, theater, film, and music. It proved to be a fascinating and very successful discussion workshop.

Sydney Gary, who came to Banbury as the first-ever Assistant Director, has been promoted to the position of Director, Research Operations, and is now based on the main Laboratory campus. Sydney took responsibility for the neuroscience programs at Banbury and, most importantly, interacted with David Stewart in developing new forms of the neuroscience lecture courses at Banbury. She did a great job and we are very sorry to lose her.

Banbury Center could not operate at the level that we did in 2007 without the outstanding efforts of many people, most especially Bea Toliver, Ellie Sidorenko, and Sydney Gary at the Conference Room, Basia Polakowski at Robertson House, and Mike Peluso and the grounds crew who look after the Banbury estate. It is only through their hard work and that of the Laboratory's Food Services and Housekeeping that Banbury can continue to fulfill its mission of being the world's best venue for serious discussions of biomedical research.

Jan Witkowski
Executive Director

MEETINGS

Epithelial Mesenchymal Transition

February 25–27

FUNDED BY **OSI Pharmaceuticals, Inc.**

ARRANGED BY **J.D. Haley,** OSI Pharmaceuticals, Inc.
A.J. Dannenberg, New York Presbyterian Hospital, Cornell

A key step in the development of cancer is the epithelial-mesenchymal transition. This reprogramming results in dedifferentiation and ultimately redifferentiation of tumor cells, so that the cells gain the ability to migrate and invade other tissues, i.e., metastasize. These invading mesenchymal-like tumor cells can then redifferentiate, leading to the reacquisition of proliferative self-renewal capacity and tumor growth at metastatic sites. These processes have a major role in the progression of cancer. Participants at this meeting reviewed the clinical and pathobiological significance of the epithelial-mesenchymal transition: the molecular signaling pathways that promote and maintain a mesenchymal-like tumor state and the animal, cell, and pathway models that might be used to further investigate the epithelial-mesenchymal transition.

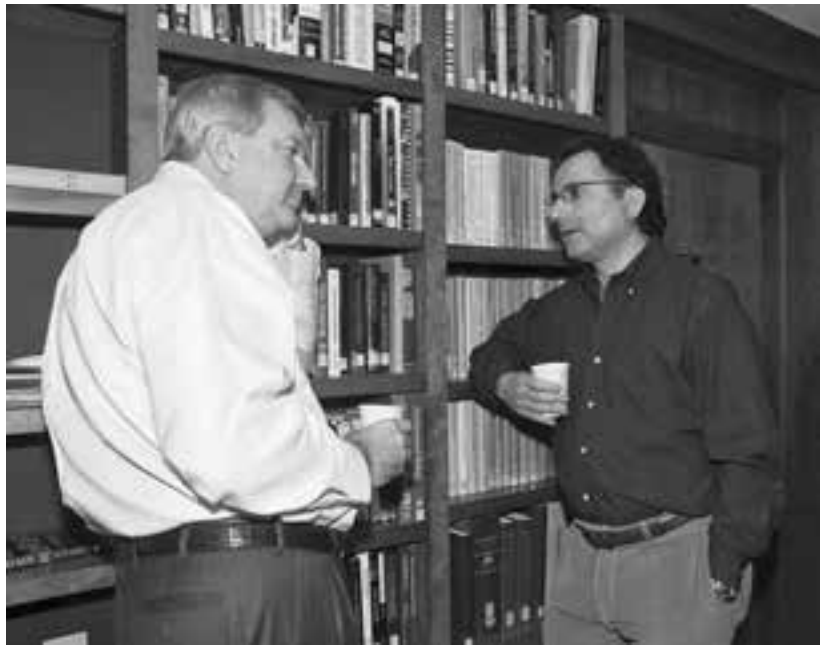
Introductory Remarks and Welcome: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
D. Epstein, OSI Pharmaceuticals, Inc., Farmingdale, New York

SESSION 1: Molecular Aspects of EMT
Chairperson: R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts

J.D. Haley, OSI Pharmaceuticals, Inc., Farmingdale, New York: EMT: A basis for the design of rational drug combinations.

R.A. Weinberg, Whitehead Institute for Biomedical Research, Cambridge, Massachusetts: Regulators of the EMT.

A. Cano, Instituto de Investigaciones Biomedicas, Madrid, Spain:



J.S. Condeelis, D. Spector

Regulation of EMT by snail and lysyl oxidase-like proteins.

A. Csiszar, Institute of Molecular Pathology, Vienna, Austria: ILEI: A cytokine essential in EMT, tumor formation, and metastasis in epithelial cells.

R. Kalluri, Harvard Medical School, Boston, Massachusetts: Targeting EMT in organ fibrosis.

SESSION 2: Cell Function and EMT
Chairperson: S. Muthuswamy, Cold Spring Harbor Laboratory

D. Radisky, Mayo Clinic Cancer Center, Jacksonville, Florida: Matrix metalloproteinase-induced EMT in breast and lung.

S. Muthuswamy, Cold Spring Harbor Laboratory: Polarity proteins regulate initiation and progression of carcinoma.

J.S. Condeelis, Albert Einstein College of Medicine, Bronx, New York: mRNA targeting is disrupted in metastatic carcinoma cells leading to EMT.

SESSION 3: Pathology and In Vivo Modeling
Chairperson: T. Brabletz, University of Erlangen-Numberg, Germany

T. Brabletz, University of Erlangen-Numberg, Germany: Malignant progression in colorectal cancer: EMT, beta-catenin, and cancer stem cells?

V. Brunton, Beatson Institute for Cancer Research, Glasgow, United Kingdom: Role of Src and FAK tyrosine kinases in tumor progression.

A. El-Naggar, The University of Texas M.D. Anderson Cancer Center, Houston, Texas: p-Src and E-cadherin have a major role in the epithelial-mesenchymal transition of head and neck squamous carcinoma.

S.M. Dubinett, University of California, Los Angeles: Inflammation: Dependent regulation of EMT in lung cancer.

Specific Issues and Priorities
Moderator: D. Epstein, OSI Pharmaceuticals, Inc.. Farmingdale, New York

A. Cano

When Is Amyloid Functional and When Is Amyloidogenesis Pathological?

March 11–14

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **J.W. Kelly,** Scripps Research Institute
K. Hsiao Ashe, University of Minnesota Medical School, Minneapolis

Amyloid has a key role in a number of degenerative disorders, but much is still unknown about the normal functions of amyloid, how the process of amyloidogenesis leads to neurodegeneration, and when amyloid is protective. Participants reviewed what is and what is not known about functional amyloid formation in a variety of tissues and contrasted this with disease-associated amyloid. Pathological amyloidogenesis (and why this process leads to tissue toxicity) was discussed in the context of amyloid, prion, and related diseases and type II diabetes.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Principles of Protein Misfolding
Chairpersons: C. Weissmann, Scripps Florida, Jupiter; **D. Walsh,** University College Dublin, Republic of Ireland

H.A. Lashuel, Swiss Federal Institute of Technology, Lausanne, Switzerland: Understanding the biochemical and structural basis of amyloid toxicity in Alzheimer's and Parkinson's disease.
M. Vendruscolo, University of Cambridge, United Kingdom: Prediction of protein aggregation propensities.
W.E. Balch, The Scripps Research Institute, La Jolla, California: Molecular and structural contributions of membrane trafficking to misfolding disease.
M. Bucciantini, University of Florence, Italy: Cell membranes as primary targets of protein aggregate cytotoxicity.
R.I. Morimoto, Northwestern University, Evanston, Illinois: Toxic protein states and the collapse of protein homeostasis.
E.R. Kandel, HHMI/Columbia University, New York: On the persistence of memory storage.
K. Si, Stowers Institute for Medical Research, Kansas City, Missouri: Does *Drosophila* Orb2 behave like a prion?

J. Collinge, B. Caughey

SESSION 2: Transmissible Prions and Amyloid Proteins
Chairpersons: R.I. Morimoto, Northwestern University, Evanston, Illinois; **G.T. Westermark,** Linkoping University, Sweden

J. Collinge, University College London, United Kingdom: Prion strains, transmission barriers, and neurotoxicity.
C. Weissmann, Scripps Florida, Jupiter: How do cells distinguish between prior strains?
S.B. Prusiner, University of California, San Francisco: Synthetic prions formed from amyloid.
B. Caughey, NIH/NIAID Rocky Mountain Laboratories, Hamilton, Missouri: Prion protein oligomerization and TSE disease.
R. Tycko, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, Maryland: Molecular structure of amyloid and yeast prion fibrils.
M. Jucker, University of Tubingen, Germany: Induction of Aβ-amyloid in transgenic mice.

S. Prusiner, J. Witkowski

SESSION 3: Functional Prions and Amyloids
Chairpersons: S.B. Prusiner, University of California, San Francisco; **W.E. Balch,** The Scripps Research Institute, La Jolla, California

R.B. Wickner, National Institutes of Health, Bethesda, Maryland: Yeast prions (PSI+) and (URE3) are disease agents in yeast.
M. Chapman, University of Michigan, Ann Arbor: Protein misfolding done right: The biogenesis of curli fibers by *E. coli*.
D.M. Fowler, The Skaggs Institute of Chemical Biology, La Jolla, California: Functional amyloid in mammals; the biogenesis of pigmentation.
M. Gebbink, University Medical Centre Utrecht, The Netherlands: Misfolded proteins, hemostasis, and immunogenicity: "The crossbeta pathway."
A.B. Bowman, Vanderbilt University Medical Center, Nashville, Tennessee: Duplication of *Atxn1* suppresses SCA1 neuropathology by decreasing incorporation of polyglutamine-expanded ataxin-1 into native complexes promoting inclusion formation.

SESSION 4: Pathological Amyloids and Misfolding Group 1
Chairpersons: S. Finkbeiner, University of California, San Francisco; **B. Caughey,** NIH/NIAID Rocky Mountain Laboratories, Hamilton, Montana

G. Hotamisligil, Harvard School of Public Health, Boston, Massachusetts: Unfolding story of diabetes, ER stress, and insulin action.
A. Dillin, Salk Institute, La Jolla, California: The genetics of age-regulated proteotoxicity: From worm to mouse.
M. Staufenbiel, Novartis Institutes for Biomedical Research Basel, Switzerland: Amyloid-associated pathological alterations in the brain of APP transgenic mice.
D. Walsh, University College Dublin, Republic of Ireland: Cell-derived Aβ oligomers and their role in Alzheimer's disease.
S. Lesne, University of Minnesota, Minneapolis: Identification and characterization of Aβ*56, a pathological Aβ assembly, causing early memory dysfunction.

SESSION 5: Pathological Amyloids and Misfolding Group 2
Chairpersons: J. Collinge, University College London, United Kingdom; **M. Staufenbiel,** Novartis Institutes for Biomedical Research Basel

P.H. Axelsen, University of Pennsylvania School of Medicine, Philadelphia: Pro-oxidant activity of amyloid-β proteins.
R. Vassar, Northwestern University, Chicago, Illinois: Multiple personalities of Aβ: Positive and negative memory functions, intraneuronal toxicity, and BACE1 elevation in AD.
S. Finkbeiner, University of California, San Francisco: Identifying species of polyglutamine proteins in situ that best predict neurodegeneration.
G.T. Westermark, Linkoping University, Sweden: Formation of intracellular IAPP-amyloid kills the β cells.

Summary
J.W. Kelly, Scripps Research Institute, La Jolla, California
K. Hsiao Ashe, University of Minnesota Medical School, Minneapolis

International Workshop on Conifer Genomics

March 18–21

FUNDED BY **Arborgen, Canadian Forest Service; European Union Evoltree; Genome British Columbia; Genome Canada; Oregon State University; Port Blakeley Tree Farms; Starker Forests; University of California, Davis; University of Georgia; University of Maine; USDA Forest Service**

ARRANGED BY **D.B. Neale,** University of California, Davis
J. Dean, University of Georgia
G.T. Howe, Oregon State University
M.S. Greenwood, University of Maine

The goals of this workshop were to advance conifer genomics research and the application of genomic tools to increase forest productivity, enhance forest health, and obtain a better understanding of all aspects of forest biology, including adaptation to environmental stresses and climate change. To this end, participants summarized the status of conifer genomics worldwide, examined the strategies pursued in other genome projects, and explored the potential of comparative genomics to advance our understanding of diverse coniferous species. There were also extensive discussions on the steps to be taken: determining priorities, coordinating research, and improving communications among genomic scientists, resource managers, ecologists, forest health specialists, research administrators, and other forest biologists.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Welcome and Meeting Objectives: D.B. Neale, University of California, Davis

SESSION 1: Why Conifer Genomics?
Chairperson: G.T. Howe, Oregon State University, Corvallis

M.S. Greenwood, University of Maine, Orono: History of conifer genomics.
G.T. Howe, Oregon State University, Corvallis: Importance of conifers: Who benefits from conifer genomics research?
R. Mangold, USDA Forest Service, Arlington, Virginia: Genomics for forest health.
D.L. Rogers, Genetic Resources Conservation Program, University of California, Davis and Center for Natural Lands Management, Fallbrook, California: Ecological genomics and conservation biology.
R.C. Purnell, Weyerhaeuser Company, Hot Springs, Arkansas: Genomics for forest industry.

D. Rogers

SESSION 2: Organization and Funding of Conifer Genomics Research
Chairperson: B. Goldfarb, North Carolina State University, Raleigh

G.S. Foster, USDA Forest Service, Washington, D.C.: Genomics and the USDA Forest Service.
A. Klein, The National Science Foundation, Arlington, Virginia and Center for Natural Lands Management, Fallbrook, California: Genomics research from competitive grants programs at NSF, DOE, and USDA.
A. Kremer, INRA UMR BIOGECO, Cestas, France: International coordination and funding for genomics research.
N. Wheeler, Molecular Tree Breeding Services, LLC, Centralia, Washington: Genomics education and outreach.
M.J. Morgan, Genome Canada, Ottawa, Canada: Genome Canada's research portfolio in forestry research.

BREAKOUT SESSION 1: Why Conifer Genomics? Organization and Funding of Conifer Genomics Research

SESSION 3: Status of Non-Conifer Genomics
Chairperson: D.B. Neale, University of California, Davis

C.H. Langley, University of California, Davis: Population genomics: Racing technologies and scrambling analysis.
J. McPherson, Baylor College of Medicine, Houston Texas: Sequencing and genomics of large and complex genomes.
R. McCombie, Cold Spring Harbor Laboratory: Maize genomics.
O. Savolainen, University of Oulu, Finland: Genomics of natural populations of *Arabidopsis*.
S.P. DiFazio, West Virginia University, Morgantown: *Populus* genomics.

SESSION 4: Status of Conifer Genomics
Chairperson: M.S. Greenwood, University of Maine, Orono

J. Mackay, University Laval, Canada: Conifer genomics in Canada.
M. Cervera, INIA-CIFOR, Madrid, Spain: Conifer genomics in Europe.
S. Cato, SCION Research, Rotorua, New Zealand: Conifer genomics in Australia and New Zealand.
D.B. Neale, University of California, Davis: Conifer genomics in the United States.

SESSION 5: Key Components of a Conifer Genomics Program
Chairperson: J. Dean, University of Georgia, Athens

J. Dean, University of Georgia, Athens: Introduction and gene discovery.
J. Bohlmann, University of British Columbia, Vancouver, Canada: Gene expression profiling, proteomics, and metabolomics.
D. Nelson, Southern Institute of Forest Genetics, Saucies, Mississippi: Mapping and genome structure.
M. Hinchee, ArborGen LLC, Summerville, South Carolina: Transgenic conifers in the private and public sectors.
K. Ritland, University of British Columbia, Vancouver, Canada: Power of comparative genomics.
S. Gonzalez-Martinez, CIFOR-INIA, Madrid, Spain: SNPs and association genetics.
J. Lee, University of California, Davis: Conifer bioinformatics.
M. Morgante, Universita' di Udine, Italy: Prospects for a conifer genome sequence.

BREAKOUT SESSION 2: Key Components of a Conifer Genomics Program

SESSION 6: Future of Conifer Genomics
Chairperson: J. Dean, University of Georgia, Athens

J. Dean, University of Georgia, Athens: Action items for conifer genomics.

BREAKOUT SESSION 3: Future of Conifer Genomics

Breakout session summaries
Large group discussion

MEETING SYNTHESIS
D.B. Neale, University of California, Davis

G. Howe

Neurobiology of Depression: From Molecules to Mood

April 1–4

FUNDED BY **Eli Lilly & Company, Memory Pharmaceuticals, Sepracor Inc., AstraZeneca Pharmaceuticals, Roche Pharmaceuticals, Wyeth Pharmaceuticals**

ARRANGED BY **R.S. Duman,** Yale University School of Medicine
G. Enikolopov, Cold Spring Harbor Laboratory
R. Hen, Columbia University, New York

Depression is a devastating illness that effects 15–20% of the population, resulting in enormous personal suffering and economic loss to society. Despite intensive research, the neurobiological mechanisms underlying the etiology and treatment of major depressive disorders have not been identified. The focus of this meeting was to undertake a comprehensive and integrated assessment of the current state of knowledge of depression research, including analysis of the genetic, molecular, and cellular determinants of mood and depression in animal models and in humans. The neurobiology of stress, which can precipitate or exacerbate depression, was discussed, as well as the behavioral consequences of stress exposure.

Welcome: S. Gary, Banbury Center, Cold Spring Harbor Laboratory,

SESSION 1: Overview/Introduction
Chairperson: H. Akil, University of Michigan, Ann Arbor

Introduction: R.S. Duman, Yale University School of Medicine, New Haven, Connecticut
G. Enikolopov, Cold Spring Harbor Laboratory
R. Hen, Columbia University, New York

R.C. Kessler, Harvard Medical School, Boston, Massachusetts: A brief overview of the epidemiology of depression.
R.R. Krishnan, Duke University Medical Center, Durham, North Carolina: Medical basis of depression.
D.S. Charney, Mount Sinai School of Medicine, New York: Novel targets for antidepressant therapeutic development: Evidence from proof of concept clinical studies.
M. Fava, Massachusetts General Hospital, Boston: How effective are antidepressant drugs?

SESSION 2: Pathophysiology
Chairperson: R.R. Krishnan, Duke University Medical Center, Durham, North Carolina

W.C. Drevets, NIH/NIMH DIRP, Bethesda, Maryland: Neuroimaging studies of depression.
S.H. Lisanby, Columbia University, New York: Targeting the neurocircuitry of depression with focal brain stimulation.
H.K. Manji, National Institute of Mental Health, Bethesda, Maryland: Cellular plasticity cascades: Genes to behavior pathways in the pathophysiology and treatment of severe mood disorders.
G. Sanacora, Yale University School of Medicine, New Haven, Connecticut: Potential contributions of the amino acid neurotransmitter systems to the pathophysiology and treatment of major depressive disorder.
D.R. Rubinow, University of North Carolina, Chapel Hill: Affective dysregulation: Lessons from reproductive neuroscience.

SESSION 3: Stress and Growth
Chairperson: H.K. Manji, National Institute of Mental Health, Bethesda, Maryland

B.S. McEwen, The Rockefeller University, New York: Stress-induced structural remodeling in brains of animals models.
R. Hen, Columbia University, New York: Neurogenesis and depression.
G. Enikolopov, Cold Spring Harbor Laboratory: Neurogenic targets of antidepressant therapies.
R.S. Duman, Yale University School of Medicine, New Haven, Connecticut: Neurotrophic factors in the pathophysiology and treatment of depression.
H. Akil, University of Michigan, Ann Arbor: Searching for novel molecules for mood disorders.

SESSION 4: Genetics and Epigenetics
Chairperson: R. Hen, Columbia University, New York

J. Gingrich, Columbia University, New York: Development contributions to affective disorders.
K.-P. Lesch, University of Wurzburg, Germany: Life stress–serotonin interaction in depression: Evidence from knockout mice and functional imaging.
K. Ressler, Emory University, Atlanta, Georgia: CRH and BDNF systems in depression: Recent genetic and molecular results.
H. Reul, University of Bristol, United Kingdom: Epigenetic mechanisms in stress-induced transcriptional activation and behavioral adaptation.
A. Kumar, University of Texas Southwestern Medical Center, Dallas: Genome-wide epigenetic and genetic changes underlying striatal plasticity associated with mood disorders.

SESSION 5: Cognition/Motivation
Chairperson: R.S. Duman, Yale University School of Medicine, New Haven Connecticut

A. Markou, University of California, San Diego: Psychostimulant drug withdrawal as an inducing condition in models of depression.
W. Carlezon, Harvard Medical School, Belmont, Massachusetts: Importance of CREB-mediated dynorphin regulation in the study and treatment of mood disorders.
J.R. Taylor, Yale University School of Medicine, New Haven, Connecticut: Murine models of depression: Linking molecules to cognitive-motivational function.

SESSION 6: Conclusions/Future Directions
Co-Chairpersons: D.S. Charney, Mount Sinai School of Medicine, New York; **H. Akil,** University of Michigan, Ann Arbor

A. Markou

New Frontiers in Studies of Nonconscious Processing

April 8–11

FUNDED BY **The Swartz Foundation**

ARRANGED BY **T.D. Wilson,** University of Virginia, Charlottesville
A. Dijksterhuis, University of Amsterdam, The Netherlands

There has been a renaissance of research on nonconscious processing, but much of this research is occurring in separate disciplines at different levels of analysis, from behavioral research to studies of neural processes. The goal of this meeting was to bring together people from different disciplines who are interested in nonconscious mental processing and its relationship to consciousness, broadly defined. Even more than is usual for a Banbury Center meeting, a very wide spectrum of disciplines was represented: social psychology, cognitive psychology, neural physiology, and philosophers. The expectation was that these participants would learn from each other, discovering that they had previously unsuspected interests in common, and that this would lead to new research directions and collaborations.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: P. Winkielman, University of California, San Diego

Introductory Remarks: T.D. Wilson, University of Virginia, Charlottesville
A. Dijksterhuis, University of Amsterdam, The Netherlands

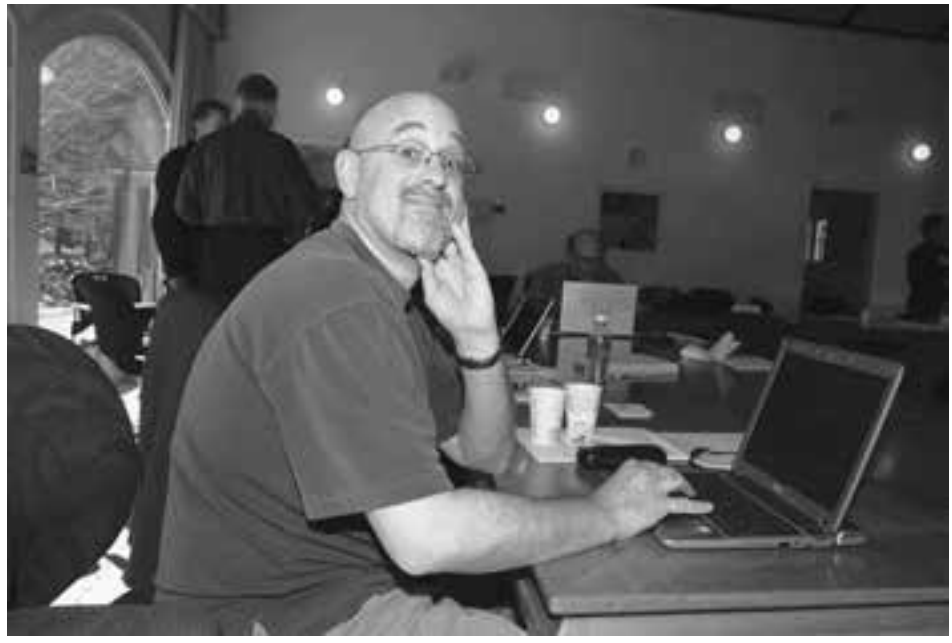
T.D. Wilson, University of Virginia, Charlottesville: The necessity of nonconscious processing.

D.M. Wegner, Harvard University, Cambridge, Massachusetts: Unconscious wellsprings of conscious will.

A. Dijksterhuis, University of Amsterdam, The Netherlands: On unconscious thought.

J.W. Schooler, University of British Columbia, Canada: Challenges of distinguishing between unconscious processes and processes that are experienced but in the absence of meta-awareness.

D. Gilbert, Harvard University, Cambridge, Massachusetts: Conscious misprediction of unconscious processes.



D. Gilbert

H. Cohen, T. Sejnowski

SESSION 2
Chairperson: D. Gilbert, Harvard University, Cambridge, Massachusetts

J. Swartz, The Swartz Foundation, East Setauket, New York: The Conscious "pop": A nonconscious processing framework for problem solving.
T. Sejnowski, The Salk Institute for Biological Studies, San Diego, California: Searching for hidden treasure unconsciously.
X.-J. Wang, Yale University School of Medicine, New Haven, Connecticut: Concept of a decision threshold in sensory-motor processes.
A.G. Greenwald, University of Washington, Seattle: Using knockout strategies to reveal conscious function.
M.N. Shadlen, HHMI/University of Washington, Seattle: Decisions, time, probability, and indeterminacy: Big ideas from small experiments.

SESSION 3
Chairperson: J.W. Schooler, University of British Columbia, Canada

R. Hassin, Hebrew University, Jerusalem, Israel: The nonconscious executive.
H. Aarts, Utrecht University, The Netherlands: Implicit motivation and regulation of goals and their pursuit.
S. Dehaene, CEA/SAC/DSV/DRM/Neurospin, Yvette, France: Human brain mechanisms of subliminal processing and conscious access.
T.L. Chartrand, Duke University, Durham, North Carolina: Nonconscious mimicry.

SESSION 4
Chairperson: C.N. Macrae, University of Aberdeen, United Kingdom

M. Ferguson, Cornell University, Ithaca, New York: On implicit evaluation.
P.S. Churchland, University of California, San Diego, La Jolla: Nonconscious imitation and valenced representations.
A. Bell, University of California, Berkeley: Emergence into consciousness viewed from the levels framework.
D.L. Schacter, Harvard University, Cambridge, Massachusetts: Priming, implicit memory, and the brain: A neuroimaging perspective.

SESSION 5
Chairperson: D.M. Wegner, Harvard University, Cambridge, Massachusetts

C.N. Macrae, University of Aberdeen, United Kingdom: When consciousness slips: Priming the absent mind.
K. Berridge, University of Michigan, Ann Arbor: Hidden brain–emotion components in desire and dread.
P. Winkielman, University of California, San Diego: Emotion and awareness.

Molecular Approaches to Pain: Translational Potential and Challenges

April 15–18

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M.E. Csete,** Emory University School of Medicine, Atlanta, Georgia
J. Prager, University of California, Los Angeles, School of Medicine

Pain remains a daunting clinical problem, and advances in understanding the molecular underpinnings of pain have not translated easily into new therapies. Much of this can be attributed to communication difficulties among the basic scientists uncovering the mechanisms of pain, the researchers developing new pain treatments, and the clinicians seeking to treat pain. Because advances in managing pain have come from diverse clinical and basic science communities, this meeting was held to foster communication among molecular biologists, neurobiologists, pharmacologists, anesthesiologists, neurologists, and neurosurgeons. Our current understanding of pain initiation and maintenance was reviewed, as well as the molecular biology of distinct pain syndromes, mechanisms of pain relief, and genetic predisposition to pain and responses to therapy.

Introductory Remarks: S. Gary, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Systems/Big Picture
Chairperson: M.E. Csete, Emory University School of Medicine, Atlanta, Georgia

J. Prager, University of California, Los Angeles, School of Medicine: Introduction and overview.
K.E. McCarson, University of Kansas Medical Center, Kansas City: Molecular mechanisms of CNS plasticity during persistent pain.
C. Sommer, Julius-Maximilians Universitat, Wuerzburg, Germany: Role of cytokines in chronic pain.
T. Samad, Harvard Medical School and Massachusetts General Hospital, Charlestown: Modulators of inflammatory pain hypersensitivity.
P.P. Mitra, Cold Spring Harbor Laboratory: Signal processing methods for LFP and EEG time series for rapid monitoring of brain state.

SESSION 2: Pathology
Chairperson: J. Prager, University of California, Los Angeles, School of Medicine

M. Garcia, University of Missouri, Columbia: Neurofilament-dependent neuronal growth and death.
A.L. Oaklander, Harvard Medical School and Massachusetts General Hospital, Boston: Major effects of minor distal nerve injuries.
A.S.C. Rice, Imperial College, London, United Kingdom: Modeling HIV-related neuropathies.
J.D. Glass, Emory University School of Medicine, Atlanta, Georgia: Mechanisms of axonal degeneration.

SESSION 3: Channels, Receptors, and Genes
Chairperson: M.E. Csete, Emory University School of Medicine, Atlanta, Georgia

J. Mao, Massachusetts General Hospital, Charlestown: Neuronal glucocorticoid receptor and neuropathic pain.
Q. Ma, Dana-Farber Cancer Institute, Boston, Massachusetts: Runx1 coordinates nocioceptor phenotypes necessary for thermal and neuropathic pain.

SESSION 4: Pharmacology/Therapies/Novel Targets
Chairperson: J. Prager, University of California, Los Angeles, School of Medicine

J. Prager, University of California, Los Angeles, School of Medicine: Neuromodulation: A multitude of targets for modulating the nervous system.
L. Mendell, Stony Brook University: Stopping pain in its tracks.
J. Kurreck, Free University Berlin, Germany: RNA interference for target validation: Investigations of the functional role of TGRPV1 in neuropathic pain.
D. Fink, University of Michigan School of Medicine, Ann Arbor: HSV-mediated gene transfer for the treatment of chronic pain.
M.E. Csete, Emory University School of Medicine, Atlanta, Georgia: Can stem cells treat pain?
R.J. Lewis, The University of Queensland, Indooroopilly, Australia: Analgesic conotoxins: Xen2174 and related stories.

SESSION 5: Clinical/Translation
Chairperson: J. Prager, University of California, Los Angeles, School of Medicine

R.J. Schwartzman, Drexel University College of Medicine, Philadelphia, Pennsylvania: Pathophysiology of CRPS.
R. Gallagher, University of Pennsylvania School of Medicine, Philadelphia: Clinical challenges for translational research.

M. Csete, J. Prager

Interactome Mapping Project for Human and Model Organisms

April 22–25

FUNDED BY **Open Biosystems and individual participants**

ARRANGED BY **M. Vidal,** Dana-Farber Cancer Institute, Boston, Massachusetts

For more than half a century, it has been conjectured that interacting macromolecules form complex systems of functionally interacting components and that the molecular mechanisms underlying most biological processes correspond to particular steady states adopted by such cellular networks. However, until recently, systems-level theoretical conjectures remained largely unappreciated, mainly because of the lack of supporting experimental data. In recent years, new large-scale high-throughput techniques are generating these data at an unprecedented rate. The participants at this meeting reviewed issues in interactome research with the goal of writing a white paper describing the goals and needs of an interactome mapping project for human and model organisms.

M. Vidal, B. Chait

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Biological Networks Properties I
Chairperson: M. Vidal, Dana-Farber Cancer Institute, Boston, Massachusetts

M. Gerstein, Yale University, New Haven, Connecticut: Understanding protein function on a genome-scale using networks.

T. Ideker, University of California, San Diego: Protein interaction networks.

Interactome meeting coffee break

SESSION 2: Cocomplex Membership Maps
Chairperson: M. Vidal, Dana-Farber Cancer Institute, Boston, Massachusetts

M. Walhout, University of Massachusetts Medical School, Amherst: Large-scale transcription factor–DNA interaction mapping using gene-centered protein–DNA interactome mapping.
J. Greenblatt, University of Toronto, Canada: Protein purification and genetic interactions to define protein complexes and functional pathways.
N. Krogan, University of California, San Francisco: Unbiased biology: Functional insights from quantitative physical and genetic interaction data sets.
T. Pawson, Mt. Sinai Hospital, Toronto, Canada: Domain-based interactions.

SESSION 3: Binary Maps
Chairperson: R.L. Finley, Wayne State University, Detroit, Michigan

S. Lalonde, Carnegie Institution of Washington, Stanford, California: Toward a comprehensive *Arabidopsis* protein interactome map: Systems biology of the membrane proteins and signalosomes.
C. Sanderson, University of Liverpool, United Kingdom: High-resolution human protein interaction networks.
R.L. Finley, Wayne State University, Detroit, Michigan: Completing the *Drosophila* protein interaction map.
E. Wanker, Max Delbrück Center for Molecular Medicine, Berlin, Germany: Automated yeast two-hybrid interaction mapping.
G. Wright, Cell Surface Signaling Laboratory, Cambridge, United Kingdom: Filling the blind spot: High-throughput identification of extracellular low-affinity interactions.

SESSION 4: Biological Networks Properties II
Chairperson: M. Vidal, Dana-Farber Cancer Institute, Boston, Massachusetts

P. Bork, EMBL, Heidelberg, Germany: Temporal aspects of protein networks.
A.-L. Barabasi, University of Notre Dame, Indiana: Human diseasome: Using protein interaction to explore human diseases.
G.F. Temple, National Human Genome Research Institute, Bethesda, Maryland: Human protein expression clones for the research community.
A. Califano, Columbia University, New York: An integrated human B-cell interactome for the dissection of lymphoid malignancies.
F. Roth, Harvard University, Boston, Massachusetts: Combining protein interactions with contextual genomic evidence to predict mammalian gene function.

SESSION 5
Chairperson: M. Vidal, Dana-Farber Cancer Institute, Boston, Massachusetts

B.T. Chait, The Rockefeller University, New York: Rapid immunoisolation of protein complexes.
L. Stein, Cold Spring Harbor Laboratory: The Reactome Database of Biological Pathways.
T. Ito, University of Tokyo, Kashiwa, Japan: Y2H and MS approaches for annotating protein interactions and modifications.
K.C. Gunsalus, New York University, New York: Probing molecular networks in *C. elegans*.

SESSION 6: Lessons from the Human Genome Project
Chairperson: M. Vidal, Dana-Farber Cancer Institute, Boston, Massachusetts

M. Vidal, Dana-Farber Cancer Institute, Boston, Massachusetts: Time for an Interactome Mapping Project!
G.M. Weinstock, Baylor College of Medicine, Houston Texas: Thoughts on the elation of Interactome and Genome projects.

SESSION 7: Summary and Future Developments
Chairperson: B.J. Wold, California Institute of Technology, Pasadena

Fragile-X Syndrome and Mechanisms of Synaptic Translation

April 29–May 2

FUNDED BY **NIH/National Institute of Mental Health (through a grant to the University of Illinois)**

ARRANGED BY **S. Warren,** Emory University, School of Medicine, Atlanta, Georgia
E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois
K. Clapp, FRAXA Research Foundation, Newburyport, Massachusetts

Fragile-X syndrome is an inherited autistic spectrum disorder resulting from the functional absence of the RNA-binding protein FMRP. FMRP normally suppresses the translation of target transcripts, and its absence results in overabundance of some of the encoded proteins. FMRP is found within and at the base of dendritic spines that are involved in synaptic plasticity, and it is believed that FMRP may have a critical role in this process. This meeting focused on the regulation of local protein synthesis at the synapse and its consequences for neurobehavioral phenotypes. Modulation of local protein translation may be one approach for rational drug design for Fragile-X syndrome. Leaders in research on processes involved in synaptic protein synthesis and those involved with FMRP biology participated, to better understand FMRP function at the synapse and the neural behavioral results of its absence.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introducing Fragile-X Patients: K. Clapp, FRAXA Research Foundation, Newburyport, Massachusetts

SESSION 1: FXS Phenotypes in Models and Man I
Chairperson: E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois

W.T. Greenough, University of Illinois, Urbana: FMRP-interacting molecules and brain phenotype.

I. Bureau, Cold Spring Harbor Laboratory: Multiple developmental circuit defects in the barrel cortex of Fragile-X mice.

S. Chattarji, National Centre for Biological Sciences, Bangalore, India: Fragile-X mental retardation protein and spine plasticity in the amygdala.

F. Bolduc, Cold Spring Harbor Laboratory, New York: Fragile-X mental retardation protein is selectively required for long-term associative memory.

P. Vanderklish, K. Huber, E. Klann

E. Klann, New York University, New York: Plasticity and behavioral phenotypes in mutant mice with altered translational control.

R.E. Paylor, Baylor College of Medicine, Houston, Texas: Modifying behavioral phenotypes of Fragile-X mouse models.

SESSION 2: FXS Phenotypes in Models and Man II
Chairperson: W.T. Greenough, University of Illinois, Urbana

S. McBride, Albert Einstein College of Medicine, Bronx, New York: Age-dependent cognitive impairment in a *Drosophila* Fragile-X model and its pharmacological rescue.

B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands: Behavioral experiments in FRAX mouse and man.

E. Berry-Kravis, Rush University Medical Center, Chicago, Illinois: Modifying behavior, biophysical measures, and cognition with lithium in FXS.

SESSION 3: Neuronal Transport
Chairperson: D.L. Nelson, Baylor College of Medicine, Houston, Texas

G.T. Bassell, Emory University, Atlanta, Georgia: The stimulating travels and functions of FMRP.

E.W. Khandjian, Laval University, Quebec, Canada: Trafficking FMRP-RNP granules in dendrites.

W. Sossin, McGill University, Montreal, Canada: Defining the multiple types of RNA particles/granules that are present in hippocampal neuron's axons.

I. Jeanne Weiler, University of Illinois, Urbana-Champaign: The cargo hypothesis of Fragile X: Applications to testing and analysis.

R.S. Zukin, Albert Einstein College of Medicine, Bronx, New York: AMPA receptor mRNA trafficking in dendrites and dysregulation in Fragile X.

SESSION 4: Functional Studies
Chairperson: B.A. Oostra, Erasmus Universiteit Rotterdam, The Netherlands

T.A. Jongens, University of Pennsylvania School of Medicine, Philadelphia: Role of the siRNA pathway in the regulation of dFMRP expression.

D.L. Nelson, Baylor College of Medicine, Houston, Texas: FXRs and FMR1 function.

R.B. Darnell, The Rockefeller University, New York: Cross-linking-IP studies on Nova.

P.W. Vanderklish, Scripps Research Institute, La Jolla, California: High-throughput proteomics: Comparison of synaptic fractions from wild-type and Fmr1 KO mice—new differences and maybe some new targets.

SESSION 5: Mechanisms of Translation
Chairperson: G.J. Bassell, Emory University, Atlanta, Georgia

S.T. Warren, Emory University School of Medicine, Atlanta, Georgia: FMRP dephosphorylation reveals an immediate-early dendritic signaling pathway.

R.D. Blitzer, Mount Sinai School of Medicine, New York: Stimulation-dependent regulation of dendritic translational capacity by mTOR.

M. Costa-Mattioli, McGill University, Montreal, Canada: Translational control of long-term synaptic plasticity and memory storage.

H. Tiedge, State University of New York, Brooklyn: Dendritic BC1 RNA in translational control mechanisms.

E.M. Schuman, California Institute of Technology, Pasadena: Regulation of local protein synthesis by synaptic transmission.

J.D. Richter, University of Massachusetts Medical School, Worcester: Translational control by CPEB.

SESSION 6: Studies of Synaptic Mechanisms and Activity
Chairperson: S.T. Warren, Emory University School of Medicine, Atlanta, Georgia

K.M. Huber, University of Texas Southwestern Medical Center, Dallas: Conservation of LTD mechanisms utilized by Gq-coupled receptors: Implications for Fragile X.

K. Broadie, Vanderbilt University and Medical School, Nashville, Tennessee: mGluR-dependent and -independent translation regulation by dFMRP in synaptic mechanisms.

C. Portera-Cailliau, University of California, Los Angeles: Imaging the origin of dendritic spine abnormalities in Fragile-X syndrome.

G. Dolen, Massachusetts Institute of Technology, Cambridge: Correction of Fragile-X syndrome in mice by reduced expression of mGluR5.

R.K.S. Wong, State University of New York, Downstate Health Science Center, Brooklyn: Divergent mGluR signaling and physiological responses.

Retreat from Reason

May 5–6

FUNDED BY **Private support**

ARRANGED BY **J. Morris,** International Policy Network, London
G. Ohrstrom, Ohrstrom Foundation Inc., New York
M. Ridley, Newcastle, United Kingdom
J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Modern technologies—especially biotechnology—are providing important benefits to society and will continue to do so. However, public perception of these technologies in both Europe and the United States, and the legal and regulatory environment in which they are developed, is increasingly hostile. As a result, access to those technologies in Europe and the United States is becoming increasingly restricted, and investments in research and development of new technologies are being curtailed or redirected to less hostile environments. The purpose of this seminar is to bring together researchers and communicators with a deep interest in the development and dissemination of new technologies. The hope is that through this discussion, a greater understanding of the causes of hostility to modern technologies will emerge, and paths forward for improving public perception and legal/regulatory environments will be identified.

Introduction to Banbury Center: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Chairperson: G. Ohrstrom, Ohrstrom Foundation, Inc., New York

Keynote Address: L.M. Silver, Princeton University, New Jersey

Panel 1: Attacks on Technology: What Have We Learned?
Chairperson: J. Morris, International Policy Network, London, United Kingdom

Panelists: **B. Ames,** Children's Hospital Oakland Research Institute, California
P. Reiter, Institut Pasteur, Paris, France
D. Taverne, House of Lords, London, United Kingdom

Panel 2: Media Representation of Technology Issues
Chairperson: J. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Panelists: **R. Bailey,** Reason Magazine, Charlottesville, Virginia
V. Postrel, Dynamist, Dallas, Texas
C. Mooney, Seed Magazine, Washington, D.C.

Chairperson: G. Ohrstrom, Ohrstrom Foundation Inc., New York

Keynote Address: V. Postrel, Dynamist, Dallas

Panel 3: Psychology of Acceptance of and Opposition to Technology
Chairperson: M. Ridley, Newcastle, United Kingdom

Panelists: **M. Crichton,** Santa Monica, California
T. Kealey, The University of Buckingham, United Kingdom
S. Dudley, Office of Management & Budget, Washington, D.C.

M. Crichton, L. Silver

M. Ridley, T. Kealey, V. Postrel

Design Principles in Biological Systems

May 6–8

FUNDED BY **The National Science Foundation**

ARRANGED BY **P.P. Mitra,** Cold Spring Harbor Laboratory
M.W. Kirschner, Harvard Medical School, Boston, Massachusetts
R.M. Murray, California Institute of Technology, Pasadena

This workshop brought together scientists with strong theoretical or mathematical backgrounds and an active interest in applying engineering principles to the study of biological systems. Discussions and presentations at the workshop focused on the premise that evolutionary solutions or designs, although not themselves engineered, may nevertheless be studied in their existing forms in the framework of theories developed for human-engineered systems. The workshop provided an opportunity for biological researchers to learn about engineering theories that may be relevant to their work and to promote collaborations by bringing interesting biological problems to the attention of engineering theorists and computer scientists.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction to Meeting: P.P. Mitra, Cold Spring Harbor Laboratory

SESSION 1: Engineering and Biology
Chairperson: R.M. Murray, California Institute of Technology, Pasadena

R.J. Full, University of California Berkeley: Challenges of an integrative systems biology.
D. Koditschek, University of Pennsylvania, Philadelphia: Evolution of a framework for development of gaits.
M. Khammash, University of California, Santa Barbara: Stochastic gene expression: New analysis tools and case studies.
R. Milo, Harvard Medical School, Boston, Massachusetts: Energy optimization and the design of photosynthesis.
D. Kleinfeld, University of California, San Diego: Topology, dynamics, and neuronal control of cortical blood flow.
C. Myers, Cornell University, Ithaca, New York: Polymorphic programming in biology and engineering.

M. Levine, D. Endy

SESSION 2: Evolution
Chairperson: P.P. Mitra, Cold Spring Harbor Laboratory

M.W. Kirschner, Harvard Medical School, Boston, Massachusetts: Physiological and evolutionary adaptation in hemoglobin.
M. Levine, University of California, Berkeley: Gene networks in animal development and evolution.
C. Queitsch, Harvard University, Cambridge, Massachusetts: Chaperone Hsp90 as a molecular mechanism of genetic and environmental canalization.
S.C. Stearns, Yale University, New Haven, Connecticut: Evolutionary principles of phenotypic design.
P. Niyogi, University of Chicago, Illinois: Computational nature of language learning and evolution.

SESSION 3: Evolution in Engineering
Chairperson: M.W. Kirschner, Harvard Medical School, Boston, Massachusetts

J. Doyle, California Institute of Technology, Pasadena: Robust and evolvable architectures.
R.M. Murray, California Institute of Technology, Pasadena: Systems engineering and architecture.

SESSION 4: Engineering and Biology (with an Emphasis on Evolution)
Chairperson: J. Carlson, Clay Mathematics Institute, Cambridge, Massachusetts

M.A. Savageau, University of California, Davis: Quantitative evolutionary design of an oxidative stress response system in human erythrocytes.
A. Sengupta, Rutgers, The State University of New Jersey, Piscataway: Geometry of parameter space in regulatory networks: Investigating measures of robustness.
H. Karten, University of California, San Diego, La Jolla: Multiple "systems" in the visual "system": An attempt to break the bottleneck.
B. Mishra, New York University, New York: Evolutionary models and systems biology.
C. Myers, University of Utah, Salt Lake City: Engineering genetic circuits.
C.C. Hilgetag, Jacobs University Bremen, Germany: Spatial organization of neural connectivity.

SESSION 5: Synthetic Biology (with Thoughts on Evolution)
Chairperson: M.E. Csete, Emory University School of Medicine, Atlanta, Georgia

C.D. Smolke, California Institute of Technology, Pasadena: Engineering RNA devices as communication and control systems.
D. Endy, Massachusetts Institute of Technology, Cambridge: Very small-scale integrated biological systems.
P.A. Silver, Harvard Medical School, Cambridge: Designing biological memory and logic.

The Brain Architecture Project

May 20–22

FUNDED BY **The W.M. Keck Foundation**

ARRANGED BY **P.P. Mitra,** Cold Spring Harbor Laboratory
L. Swanson, University of Southern California, Los Angeles
H. Breiter, Massachusetts General Hospital, Charlestown
J. Doyle, California Institute of Technology, Pasadena
C. Allen, Cold Spring Harbor Laboratory

This Project is a new collaborative effort in human neuroanatomical research supported by the W.M. Keck Foundation. The initial goal of the project is to produce a draft of the "connectivity matrix" of the human brain, along with analytical and visualization tools. The main focus was collation and integration of human neuroanatomical information in the existing literature into a comprehensive database. This was the first in what will be a series of annual meetings intended to promote the development of useful resources for the neuroscience research and clinical communities, as well as to receive valuable feedback and input from collaborators and invited external advisors. Progress to date was reviewed, and there were sessions focused on the reconciliation of classical neuroanatomical and MRI morphometry-based systems of nomenclature.

Opening Session: Introducing the Brain Architecture Project

Introduction of Project and Goals: P.P. Mitra, Cold Spring Harbor Laboratory

Overview of Progress and Meeting Agenda; Introductions to Project Members and Meeting Participants: C.B. Allen, Cold Spring Harbor Laboratory

Additional Remarks: H. Breiter, Massachusetts General Hospital, Charlestown
L.W. Swanson, University of Southern California, Los Angeles

Introductory Remarks: S. Gary, Banbury Center, Cold Spring Harbor Laboratory

N. Schiff, C. Allen, D. Herrera

SESSION 1: Related Projects
Chairperson: G. Burns, University of Southern California, Los Angeles

D.M. Bowden, University of Washington, Seattle: Terminological needs of a portal to neuroscience on the Web.

L.W. Swanson and M. Bota, University of Southern California, Los Angeles: Classical nomenclature and the Brain Architecture Management System (BAMS).

C.B. Allen, Cold Spring Harbor Laboratory: Overview of the CoCoMac database.

C.C. Hilgetag, Jacobs University Bremen, Germany: Perspectives on CoCoMac from an end-user.

S. Habeer, University of Rochester, New York: Three-dimensional models of projection pathways in the macaque brain.

D. Kennedy, N. Makris, and H. Breiter, Massachusetts General Hospital, Charlestown: MRI-based neuroanatomic parcellation and labeling systems: Implications for broad, community-based utility.

SESSION 2: Proposed Scenarios for Nomenclature Reconciliation
Chairperson: C.B. Allen, Cold Spring Harbor Laboratory

Direct comparison of MRI parcellation systems (BAP members)
Reconciliation Scenarios (BAP members)
Moderated Discussion

SESSION 3: Networks
Chairperson: C.B. Allen, Cold Spring Harbor Laboratory

P.P. Mitra, Cold Spring Harbor Laboratory: Structure and dynamics of networks: An informal review of models and analysis tools.

SESSION 4: Coordinating with Atlasing Projects
Chairperson: H. Barbas, Boston University, Massachusetts

E.G. Jones, University of California, Davis: Brain atlases and terminology.
D.C. Van Essen, Washington University School of Medicine, St. Louis, Missouri: Cortical partitioning and connectivity analyzed using surface-based atlases and approaches.
A.W. Toga, Laboratory of Neuro Imaging, Los Angeles, California: Multisubject, multisite brain atlasing projects: Past and future experiences.

SESSION 5: Advisor Input: Open discussion with advisors on overall project goals and plan.

SESSION 6: White Paper Input: Open discussion to voice the needs of neuroanatomy and connectivity research.

The Laboratory Diagnosis of Lyme Disease

September 9–12

FUNDED BY **Centers for Disease Control and Prevention and NIH—National Institute of Allergy and Infectious Diseases**

ARRANGED BY **P.J. Baker,** NIAID, National Institutes of Health, Bethesda, Maryland
R.J. Dattwyler, New York Medical College, Valhalla
B.J.B. Johnson, CDC, DVBID, Fort Collins, Colorado

Lyme disease continues to be a difficult infection to diagnose and treat, even 25 years after the discovery that it was caused by a spirochete transmitted by tick bites. Banbury Center has been the venue for Lyme disease discussion meetings almost annually since 1991, and it is fascinating to see how the topics for meetings have cycled through basic research, diagnosis, and treatment. This year, the meeting returned to the problems and possible solutions of diagnosis. This is clearly a matter of the greatest importance in infectious diseases. The correct diagnosis must be made as early as possible so that the correct treatment can be administered as soon as possible.

M. Gomes-Solecki, R. Dattwyler

Introductory and Welcoming Remarks

J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
P.J. Baker, NIAID, National Institutes of Health, Bethesda, Maryland

Goals for the Conference

B.J.B. Johnson, CDC, DVBID, Fort Collins, Colorado
R.J. Dattwyler, New York Medical College, Valhalla

SESSION 1: Serodiagnosis of Lyme Disease in Clinical Practice I
Chairperson: A.C. Steere, Massachusetts General Hospital, Boston

B.J.B. Johnson, CDC, DVBID, Fort Collins, Colorado: Initial efforts to standardize the serodiagnosis of Lyme disease: Rationale, history, and retrospective studies.
M. Aguero-Rosenfeld, Westchester County Medical Center, Valhalla, New York: Lyme serology: Two-tier revisited.
M.J. Binnicker, Mayo Clinic, Rochester, Minnesota: Evaluation of automated western blot processing, scanning, and interpretive systems in the serologic diagnosis of Lyme disease.
A.C. Steere, Massachusetts General Hospital, Boston: Prospective study of serologic tests for Lyme disease.
J.A. Branda, Massachusetts General Hospital, Boston: A new approach to antibody testing in the diagnosis of Lyme disease.

SESSION 2: Serodiagnosis of Lyme Disease in Clinical Practice 2
Chairperson: S. O'Connell, Southampton General Hospital, United Kingdom

B. Wilske, University of Munich, Germany: Diversity of *B. burgdorferi* sensu lato in Europe and implications for diagnosis of Lyme borreliosis.
S. O'Connell, Southampton General Hospital, United Kingdom: Lyme diagnostics and clinical advice provision in the U.K.: Experience of a reference laboratory.
H. Hofmann, University of Munich, Germany: Serological follow up in patients with early and late Lyme borreliosis: Densitometric evaluation of a new Line Blot compared to quantitative ELISA.
P.J. Krause, Connecticut Children's Medical Center, Hartford: Absence of serologic cross-reactivity between *B. burgdorferi* and *B. microti*.
A.R. Marques, LCI, NIAID, National Institutes of Health, Bethesda, Maryland: Evaluation of CSF in the diagnosis of neuroborreliosis.
C.P. Quinn, CDC, Atlanta, Georgia: Development and validation of quantitative serological assays.

SESSION 3: VosE and C6 Peptide
Chairperson: M.T. Philipp, Tulane University Health Sciences Center, Covington, Louisiana

M.T. Philipp, Tulane University Health Sciences Center, Covington, Louisiana: Comparative antigenicity of VlsE, C6, and other VlsE invariant regions and domains.
G.P. Wormser, New York Medical College, Valhalla: C6 vs. two-tier testing: A multicenter study.
S.J. Wong, New York State Department of Health, Albany: Comparison of Athena Multi-Lyte *B. burgdorferi* VlsE1 IgG+ pepC10 IgM test system to two-tier Lyme serology.
M.J. Gomes-Solecki, New York Medical College, Valhalla: Multiantigenic peptide assay for the serodiagnosis of Lyme disease.
P. Lahdenne, University of Helsinki, Finland: Improved serodiagnosis of Lyme disease with recombinant protein variants from different borrelial genospecies.

SESSION 4: Future Directions
Chairperson: A.G. Barbour, University of California, Irvine

R.B. Porwancher, Infectious Disease Consultants, P.C., Mercerville, New Jersey: Improving Lyme disease diagnosis through bioinformatics.
I. Schwartz, New York Medical College, Valhalla: Host-gene expression as a diagnostic test for *B. burgdorferi* infection.
P.L. Felgner, University of California, Irvine: Whole proteome microarrays for serodiagnostic antigen discovery.
A.G. Barbour, University of California, Irvine: Discovery and rediscovery of *B. burgdorferi* antigens: A genome-wide array approach.
J. Carroll, University of Pittsburgh, Pennsylvania: Paralogous gene family 54 and proteomic approaches to serodiagnosis.

SESSION 5: Future Directions II
Chairperson: S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark

A.R. Marques, LCI, NIAID, National Institutes of Health, Bethesda, Maryland: B-cell-attracting chemokine CXCL 13 in patients with post-Lyme disease syndrome.
M. Eshoo, Ibis Biosciences, Carlsbad, California: Identifying unknown pathogens by mass spectrometry.
S.E. Schutzer, UMDNJ–New Jersey Medical School, Newark: Proteomics of Lyme disease.

Special Presentation: Populist syndromes: Toxic mold and chronic Lyme disease
M. Edesess, International Development Enterprises, Lakewood, Colorado

SESSION 6: Regulation of Diagnostic Testing
Chairperson: B.J.B. Johnson, CDC, Fort Collins, Colorado

S. Hojvat, FDA/CDRH/OIVD, Rockville, Maryland: FDA regulation of Lyme disease serological tests for the detection of *B. burgdorferi* antibodies.

SESSION 7: Recommendations of the Conferees

Roundtable: Session chairs and conveners

Champalimaud Foundation: Neuroscience

September 13–14

FUNDED BY **The Champalimaud Foundation**

ARRANGED BY **A. Damasio,** University of Southern California, Los Angeles
J.D. Watson, Cold Spring Harbor Laboratory

The Champalimaud Foundation has decided to establish an international institute of research and clinical practice, focused on cancer and neurological illnesses. Initial plans are under way, including the formation of partnerships with a number of outstanding international universities, teaching hospitals, and research institutions. As part of these initial plans, the Foundation held two discussion workshops at Banbury, the first on neuroscience and the second on cancer (see next page). The following critical questions were put to the invited experts: What kind of science should be pursued? What should the balance be between clinical work and basic research? What new technologies or concepts can best be developed and applied?

Introduction: A. Damasio, University of Southern California, Los Angeles
J.D. Watson, Cold Spring Harbor Laboratory

Chairperson of Meeting: A. Damasio, University of Southern California, Los Angeles

SESSION 1: Current Plans from Champalimaud Foundation I

Neuroscientists (led by Z. Mainen) and reaction from the Group of Advisors

SESSION 2: Current Plans from Champalimaud Foundation II

SESSION 3: Building a Successful Neuroscience Program: Useful Paths and Pitfalls

SESSION 4: Final Discussion and Recommendations

J. Watson, L. Beleza, A. Damasio

Z. Mainen

Champalimaud Foundation: Cancer Research

September 15–16

FUNDED BY **The Champalimaud Foundation**

ARRANGED BY **C. Caldas,** Cancer Research UK, Cambridge
J.D. Watson, Cold Spring Harbor Laboratory

Introduction—The Champalimaud Foundation Cancer Program
J.D. Watson, Cold Spring Harbor Laboratory
C. Caldas, Cancer Research UK, Cambridge

Chairperson of Meeting: C. Caldas, Cancer Research UK, Cambridge

SESSION 1: Cancer Genomes and Epigenomes in the Era of Very Cheap Sequencing

Discussion Leaders:
B.J. Ponder, Cancer Research UK, Cambridge: Germ-line genomics.
M. Loda, Dana-Farber Cancer Institute, Boston, Massachusetts: Somatic genomics.

SESSION 2: Cancer Microenvironment: Angiogenesis and More

Discussion Leaders:
J. Folkman, Children's Hospital, Boston, Massachusetts: Angiogenesis.
D. Hanahan, University of California, San Francisco: Tumor stroma.

SESSION 3: Cancer Imaging: Seeing It All Noninvasively

Discussion Leader:
R. Blasberg, Memorial Sloan-Kettering Cancer Center, New York: State of the art in molecular imaging.

SESSION 4: Cancer Models: Building Mice with "Humanized" Cancer

Discussion Leaders:
S. Lowe, Cold Spring Harbor Laboratory: RNAi in mice.
D.A. Tuveson, Cancer Research UK, Cambridge: Engineering mice with "human-like" tumors.

SESSION 5: Experimental Cancer Therapeutics: The Way Ahead

Discussion Leaders:
C.J. Marshall, Institute of Cancer Research, London, United Kingdom: A systems approach to targeting kinase pathways.
S. Aparicio, BC Cancer Research Centre, Vancouver, Canada: Image-based high-throughput "synthetic-lethal" screens.

SESSION 6: Cancer Stem Cells: Does It Matter?

Discussion Leaders:
C. Eaves, BC Cancer Research Centre, Vancouver: Breast stem cells and the molecular phenotypes of breast cancer.
P.G. Pelicci, European Institute of Oncology, Milan, Italy: PML and leukemia stem cells.

SESSION 7: Final Discussion: The Ideal Cancer Research Institute—Balancing Fundamental and Applied Research

Discussion Leaders:
B.J. Ponder, Cancer Research UK, Cambridge
P. Marks, Memorial Sloan-Kettering Cancer Center, New York

J. Folkman, J. Botelho, D. Hanahan, J. Watson

Drug Discovery, Biomarkers, and Clinical Trials for ALS

September 23–26

FUNDED BY **Greater New York Chapter of the ALS Association**

ARRANGED BY **E.P. Pioro,** The Cleveland Clinic, Ohio
M.D. Cudkowicz, Harvard Medical School, MGH, Boston, Massachusetts
L. Bruijn, The ALS Association, Palm Harbor, Florida
N. Kayadjanian, The ALS Association, San Diego, California

As we understand more about the underlying mechanisms involved in cell death in ALS, several new targets for therapeutic development have been identified. Some of the key challenges associated with the development of therapies for ALS include the degree of cell death at the time of diagnosis and the length of clinical trials required to determine whether the drug is effective in slowing disease progression. This workshop evaluated some of the exciting new areas and opportunities for drug discovery and development, and explored the development of biomarkers and clinical trial design.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Drug Discovery Efforts in ALS
Chairperson: J.D. Rothstein, The Johns Hopkins University School of Medicine, Baltimore, Maryland

L. Bruijn, The ALS Association, Palm Harbor, Florida: Introduction.
R. Pacifici, MRSSI/CHDI, Inc., Los Angeles, California: Drug discovery and development in other orphan diseases such as Huntington's disease: Opportunities and challenges.
P.T. Lansbury, Link Medicine, Corporation, Cambridge, Massachusetts: Therapeutic strategies for slowing ALS progression.
J.M. McCall, PharMac LLC, Kalamazoo, Michigan: Drug discovery and development: Challenges and opportunities.
J.D. Rothstein, The Johns Hopkins University School of Medicine, Baltimore, Maryland: Modulation of astroglial function in ALS: Small-molecule discovery, relevant biomarkers and PET.

SESSION 2: High-throughput Screening
Chairperson: R. Pacifici, MRSSI Inc., Los Angeles, California

D.F. Fischer, BioFocus DPI, Leiden, The Netherlands: High-throughput target and drug discovery in human stem-cell-derived motor neurons.
J. Staunton, CombinatoRx, Inc., Boston, Massachusetts: Platform for testing combination therapies in high-throughput cell-based assays.

SESSION 3: Delivery Systems
Chairperson: M.D. Cudkowicz, Harvard Medical School, MGH, Boston, Massachusetts

D. Drummond, Hermes Biosciences, Inc., South San Francisco, California: Immunotargeted lipid nanocarriers for small-molecule and nucleic acid therapeutics.
Z. Xu, University of Massachusetts Medical School, Worcester: RNAi therapy for ALS.
T.M. Miller, Washington University School of Medicine, St. Louis, Missouri: Antisense and ALS.

General Discussion
M.D. Cudkowicz, Harvard Medical School, MGH, Boston, Massachusetts

P. Kauffman, J.M. McCall

SESSION 4: Clinical Trials in ALS
Chairperson: P. Kaufmann, The SMA Clinic, Columbia University Medical School, New York

M.D. Cudkowicz, Harvard Medical School, Massachusetts General Hospital, Boston: Overview.
W.W. Bryan, Biologics Consulting Group, Inc., Rockville, Maryland: Is ALS one disease or many different diseases?
B. Ravina, Strong Health Medical Center, Rochester, New York: Clinical trial designs in Parkinson's disease.
S. Wieland, CytRx Corporation, Los Angeles, California: Clinical trial design in ALS for product approval.
B. Levin, Columbia University, New York: Sequential statistical designs for selecting from competing therapies.
D.A. Schoenfeld, Harvard School of Public Health, Boston, Massachusetts: Design considerations for trials of ALS therapies.

SESSION 5: Biomarkers I
Chairperson: N. Kayadjanian, The ALS Association, San Diego, California

E.P. Pioro, The Cleveland Clinic, Ohio: Overview.
M. Benatar, Emory University, Atlanta, Georgia: Strategies for presymptomatic biomarker identification in familial ALS.
M. Strong, UH-LHSC, London, Ontario, Canada: FTD-TDP43 and other clues as biomarkers/pathology.
J.S. Paulsen, University of Iowa, Iowa City: Lessons from other diseases: Huntington's disease.
M. Lowe, The Cleveland Clinic Foundation, Ohio: Imaging techniques.
J.M. Shefner, State University of New York, Upstate Medical University, Syracuse: Physiological outcome measures in ALS clinical trials.

General Discussion:
E.P. Pioro, The Cleveland Clinic, Ohio

SESSION 6: Biomarkers II
Chairperson: E.P. Pioro, The Cleveland Clinic, Ohio

R.H. Brown, Massachusetts General Hospital, Charlestown: Overview.
R. Bowser, University of Pittsburgh School of Medicine, Pennsylvania: Use of biomarkers to identify therapeutic targets and monitor disease progression.
B. McCreedy, Metabolon, Inc., Research Triangle Park, North Carolina: Metabolomic approach to biomarkers for ALS.

SESSION 7: Animal Models
Chairperson: L. Bruijn, The ALS Association, Palm Harbor, Florida

D.S. Howland, High Q Foundation, New York: Standardization of preclinical testing in mouse models of ALS and HD: A pipeline for targets and compounds.

D.F. Fischer

Panel Discussion: Challenges for Preclinical and Clinical Development of Therapies for ALS
Chairperson: M.D. Cudkowicz, Harvard Medical School, MGH, Boston, Massachusetts
Panelists: **J. McCall,** PharMac LLC, Kalamazoo, Michigan: Drug development.
B. Ravina, University of Rochester, New York: Clinical trials.
R.H. Brown, Massachusetts General Hospital, Charlestown: All aspects.
W.W. Bryan, Biologics Consulting Group Inc., Rockville, Maryland: Regulatory issue.
D.S. Howland, High Q Foundation, New York: Animal models.

Wrap Up and Closing Remarks
L. Bruijn, The ALS Association, Palm Harbor, Florida

Science: Get It Across!

September 28–October 3

FUNDED BY **Boehringer Ingelheim Fonds Foundation for Basic Research in Medicine**

ARRANGED BY **H. Fröhlich,** Boehringer Ingelheim Fonds, Heidesheim, Germany
C. Walther, Boehringer Ingelheim Fonds, Heidesheim, Germany

The Boehringer Ingelheim Foundation returned to the Banbury Center for their biannual fellows meeting in North America. In addition to providing training for their fellows, the Foundation very generously supported a special lecture by a visiting young scientist, given in Grace Auditorium and open to all CSHL scientists. The first recipient of this honor was Jennifer A. Zallen, Head of the Morphogenesis and Polarity Laboratory at Memorial Sloan-Kettering Institute, who spoke on "Shaping the embryo: Cellular dynamics in development."

Opening Remarks: H. Fröhlich, Boehringer Ingelheim Fonds, Heidesheim, Germany

Introduction: C. Walther, Boehringer Ingelheim Fonds, Heidesheim, Germany

Speakers

W. Wells, Global Alliance for TB Drug Development, New York: Basic lecture on writing techniques and how to structure papers.
H. Ploegh, Whitehead Institute, Cambridge, Massachusetts: What makes success in science?
B. Tansey, Cold Spring Harbor Laboratory: Representation of scientific information: Graphic and rhetoric.
B. Tansey, Cold Spring Harbor Laboratory: Group A starts the graphic assignment and Group B delivers 10-minute-presentation.
B. Tansey, Cold Spring Harbor Laboratory: Review of the videotaped presentations (Group B).
B. Tansey, Cold Spring Harbor Laboratory: Group B starts the assignment and Group A delivers 10-minute-presentation.
B. Tansey, Cold Spring Harbor Laboratory: Review of the videotaped presentations (Group A).
J.A. Zallen, Memorial Sloan-Kettering Cancer Center, New York: Shaping the embryo. Cellular dynamics in development.

Sammis Hall in the fall

Genetics of Crop Domestication

October 14–17

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **M.D. Purugganan,** New York University
E.S. Buckler, Cornell University, Ithaca, New York

The development of domesticated species was a pivotal event in the rise of human civilizations and has served as a metaphor for the evolution of new taxa. In recent years, geneticists and plant scientists have made significant advances in identifying genes associated with domestication, as well as using molecular data to explore the evolutionary process of domestication. Archaeologists have also made strides in unraveling the record of crop and farm animal use in many sites across the world and have begun to explore the cultural, ecological, and evolutionary patterns associated with domestication. As for many Banbury meetings, the organizers ensured an interesting meeting by having participants from varied backgrounds—genomic scientists, molecular biologists, plant breeders, evolutionary geneticists, and archaeologist—in this case, to explore the origins and evolution of domesticated plant and animal species.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Plant and Animal Domestication and the Archaeological Record
Chairperson: J.F. Wendel, Iowa State University, Ames

D.Q. Fuller, University College London, United Kingdom:
Progress and challenges in the Archaeobotany of Asian rice: Spikelet bases, immature harvests, and inferring domestication process.

B.D. Smith, Smithsonian Institution, Washington, D.C.:
Combing genetics and archaeology in documenting the early

history of four crop plants in the Americas: Bottle gourd, squash, maize, and sunflower.

M.A. Zeder, Smithsonian Institution, Washington, D.C.: Defining domestication: How advances in genetics and archaeology are reshaping our understanding of domestication and its origins.

G. Wilcox, National Centre for Scientific Research, Saint-Paul-le-Jeune, France: Origins of cultivation and domestication of cereals and pulses: Evidence from Near Eastern archaeological sites.

P. Gepts

SESSION 2: Origins of Agricultural Species: Molecular Data
Chairperson: D.Q. Fuller, University College London, United Kingdom

P.L. Morrell, Monsanto Company, Chesterfield, Missouri: Genetic evidence for a second domestication of barley (*Hordeum vulgare*) east of the Fertile Crescent.

P. Gepts, University of California, Davis: A Phaseoulus perspective on crop domestication.

D.G. Bradley, Trinity College Dublin: Genetic hoof prints; genetic insight into bovine domestications.

G. Larson, Uppsala University Biomedical Center, Sweden: Breaking down genomic barriers: How domestication affects an organism's ability to hybridize with multiple wild species.

R.K. Wayne, University of California, Los Angeles: Dog origins, domestication, and evolution.

J.F. Wendel, Iowa State University, Ames: Jeans, genes, and parallel domestication of cultivated cottons.

P. Tiffin, University of Minnesota, St. Paul: Population genetics of the progenitor: Geographic structure and diversity in teosinte.

SESSION 3: Selection in Domesticated Genomes
Chairperson: P. Tiffin, University of Minnesota, St. Paul

C.D. Bustamante, Cornell University, Ithaca, New York: Statistical methods for inferring demographic history of domesticated plant and animal species from SNP genotype data: Preliminary data, potential pitfalls, and possible solutions.

Y. Kim, Arizona State University, Tempe: The pattern of selective sweeps in derived populations.

A.L. Caicedo, University of Massachusetts, Amherst: Genome-wide patterns of nucleotide polymorphism in domesticated rice.

M.D. Purugganan, New York University, New York: Nature of selection in the rice genome.

S.I. Wright, York University, Toronto, Canada: Demographic history and selection during maize domestication.

T. Brown, University of Manchester, United Kingdom: Using computer simulations to test models of crop origins.

SESSION 4: Genetic Architecture and Molecular Genetics of Domestication
Chairperson: A.L. Caicedo, University of Massachusetts, Amherst

T. Sang, Michigan State University, East Lansing: Genetics and phylogenetics of rice domestication.

S. McCouch, Cornell University, Ithaca, New York: Emerging story of rice domestication.

J.M. Burke, University of Georgia Plant Biology, Athens: Genetics and the domestication of sunflower.

W. Powell, National Institute of Agriculture Botany, Cambridge, United Kingdom: Evolution and domestication of barley and wheat: New insights from population-based resequencing of candidate genes.

E.S. Buckler, Cornell University, Ithaca: Genetic architecture of maize trait variation.

L. Andersson, Uppsala University, Sweden: Molecular characterization of trait loci gives new insight in chicken domestication.

K.M. Olsen, Washington University in St. Louis, Missouri: Molecular evolution of an adaptive cyanogenesis polymorphism in white clover.

N. Weeden, Montana State University, Bozeman: Genetic basis of morphological and physiological changes associated with the domestication of pea, *P. salivum L*.

Meeting Wrap Up and Discussion of Future Research

Protecting Public Trust in Immunization

October 17–19

FUNDED BY **Albert B. Sabin Vaccine Institute, with support from the Robert Wood Johnson Foundation and Autism Speaks**

ARRANGED BY **L.Z. Cooper,** Sabin Vaccine Institute, New York
H. Larson, Harvard Center for Population & Development, Cambridge, Massachusetts
S.L. Katz, Duke University Medical Center, Durham, North Carolina

Vaccine preventable diseases (with few exceptions) are at an all time low in the United States. This success reflects the biologic effectiveness of specific vaccines, sound public policy, implementation in delivering vaccines to target audiences, and a history of high levels of public trust in vaccine safety and efficacy. This trust is an expression of a special social contract that is key to the success of immunization programs. However, we cannot be complacent in assuming trust in public health recommendations, and, indeed, a significant number of parents have serious concerns about safety. Antivaccine activists are gaining momentum. Given these recent trends, it is critical to take a closer look at public trust in vaccines. This discussion meeting reviewed the status of public trust in immunization, clarified its strengths and weaknesses, identified strategies to increase trust, and made recommendations to stakeholders in the "vaccine endeavor."

Keynote Speaker: H.R. Shepherd, Sabin Vaccine Institute, New Canaan, Connecticut

Introduction of Speaker: Robert Wright, Autism Speaks, New York

SESSION 1: A Frame of Reference
Chairperson: S.L. Katz, Duke University Medical Center, Durham, North Carolina

L.Z. Cooper, Sabin Vaccine Institute, New York: Why Sabin convened this colloquium?
D.G. Salmon, National Vaccine Program, DHHS, Washington, D.C.: Government roles in protecting public trust in immunization.
D. Pineda, Immunizations for Public Health, Galveston, Texas: Words matter: Risk communication and public trust.

Group General Discussion and charge to Breakout Group A: What should be key features immunization programs to sustain public trust of individuals to be served?

Group's Report

SESSION 2: Painful Examples: Evolution of Distrust and Lessons to be Learned
Chairperson: L.Z. Cooper, Sabin Vaccine Institute, New York

Discussion: Autism—The MMR Questions Evolution of Chimerical Concerns
Panel: N. Halsey, The Johns Hopkins University School of Hygiene & Public Health, Baltimore, Maryland
G. Nowak, Centers for Disease Control and Prevention, Atlanta, Georgia
S. Bernard, SafeMinds, Tyrone, Georgia
A. Shih, Autism Speaks, New York
M. McCormick, Harvard School of Public Health, Boston, Massachusetts: A special perspective: The IOM-ISRC experience—Trust, a steep hill.

SESSION 3: Global Issues: Biologic and Sociocultural/Political Complexities Challenge Public Trust

A. Polio and Beyond, Tetanus and Hepatitis Vaccines
Chairpersons: A. Bentsi-Enchill, World Health Organization, Geneva, Switzerland
H. Larson, Harvard Center for Population & Development, Cambridge, Massachusetts
N. Khuri-Bulos, Jordan University Hospital, Amman
K. Hartigan-Go, The Zuellig Foundation, Makati City, Philippines

B. Other Challenges, Including Balancing National vs. International Interests
Chairperson: S.L. Katz, Duke University Medical Center, Durham, North Carolina

J.D. Grabenstein, Merck Vaccines & Infectious Diseases, West Point, Pennsylvania: HPV, a new primary audience, different challenges.
D.R. Johnson, Sanofi Pasteur, Swiftwater, Pennsylvania: Ups, downs, and ups with totavirus vaccine.
L. Sullivan, Morehouse School of Medicine, Atlanta, Georgia: A broader perspective on public trust and immunization.

Brief Comments from the "Communicators"
Chairperson: C.D. DeAngelis, Journal of the American Medical Association, Chicago, Illinois
Panel: C. Cole, Sesame Workshop, New York
L. McNeill, Food and Drug Administration, Rockville, Maryland
B. Mulach, NIAID, National Institutes of Health, Bethesda, Maryland
G. Nowak, Centers for Disease Control and Prevention, Atlanta, Georgia
D. Pineda, Immunizations for Public Health, Galveston, Texas

Breakout Group B
What are the lessons to be learned from these recent challenging examples? Reflect on both the processes of communication and the specific content of the information.

Breakout Group B Reports and Open Discussion

Breakout Group C
Since immunization science provides content essential for communication and building public trust, what are solutions for enhancing immunization safety science?

Group's Report and Group Discussion: Selecting most important solutions

Open Comments: What have we missed? What will you take away? What will you do toward protecting public trust?
Panel: L.Z. Cooper, Sabin Vaccine Institute, New York
S.L. Katz, Duke University Medical Center, Durham, North Carolina
H. Larson, Harvard Center for Population & Development, Cambridge, Massachusetts: Wrap up by the organizers, including next steps and final group discussion.

L. Gordon, I. Sullivan

Microbial Forensics: Enduring Research Pathways

October 21–24

FUNDED BY **U.S. Department of Homeland Security and individual participants**

ARRANGED BY **S. Schutzer,** UMDNJ–New Jersey Medical School, Newark
B. Budowle, Federal Bureau of Investigation Laboratory, Quantico, Virginia

A major thrust of microbial forensics is the continuing quest for technologies and strategies that can improve the characterization of samples. At present, these fall into two major categories: nucleic-acid-based assays and chemical assays. The former enable association (or elimination) of a pathogen with specific sources using genetic information, and the latter provides information on the processes used to grow, stabilize, and/or disseminate the agent. In both areas, technologies are needed for rapid, high-sensitivity, and highly specific analysis of pathogens in limited and complex samples. Participants sought to identify the requirements of such technologies, to better guide the community in research and development and administrators in selecting what to support.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Overview
Chairperson: S. Schutzer, UMDNJ–New Jersey Medical School, Newark

B. Budowle, Federal Bureau of Investigation, Quantico, Virginia: Microbial forensics and enduring technologies.
P.T. Pesenti, U.S. Department of Homeland Security, Washington, D.C.: DHS program for long-term needs to support field and goals.
J.P. Burans, U.S. Department of Homeland Security, Frederick, Maryland: National labs and NBFAC efforts in enduring technology.

SESSION 2: Proteomics
Chairperson: B.L. Marrone, Los Alamos National Laboratory, New Mexico

J.N. Adkins, Pacific Northwest National Laboratory, Richland, Washington: Mass spectrometry identification of forensic material.
K.L. Wahl, Pacific Northwest National Laboratory, Richland, Washington: Mass spectrometry tool for identifying nonprotein non-DNA signatures.

SESSION 3: DNA Typing Technologies
Chairperson: J.J. Dunn, Brookhaven National Laboratory, Upton, New York

W.R. McCombie, Cold Spring Harbor Laboratory: Comparison of high-throughput sequencing capabilities.
M. Srinivasan, 454 Life Science, Bradford, Connecticut: 454 sequencing.
M.R. Furtado, Applied Biosystems, Foster City, California: Solid and multiplex SNP technologies.

SESSION 4: Threat Assessment and Legal Issues
Chairperson: P.J. Jackson, Los Alamos National Laboratory, New Mexico

J. Smith, Federal Bureau of Investigation, Washington, D.C.: Credible threat assessments: Bases and practices.
R.P. Harmon, Alameda County District Attorney's Office, Oakland, California: Legal issues for technologies, routine, new, and one-of-a-kind, Daubert.

SESSION 5: Improving Template Quality for Downstream Assays
Chairperson: R.P. Harmon, Alameda County District Attorney's Office, Oakland, California

M. Eshoo, Ibis Bioscience, Carlsbad, California: Whole-genome amplification.
T.E. Evans, New England BioLabs, Inc., Ipswich, Massachusetts: DNA repair.
S. Schutzer, UMDNJ–New Jersey Medical School, Newark: Integration of proteomics and genomics.
S.P. Velsko, Lawrence Livermore National Laboratory, California: Analytical technologies for nonbio signatures.

SESSION 6: Interpretation
Chairperson: S.A. Morse, Centers for Disease Control and Prevention, Atlanta, Georgia

B. Budowle, Federal Bureau of Investigation, Quantico, Virginia: Needs for interpretation of results.
M.A. Feinberg and J. Bannan, Federal Bureau of Investigation, Quantico, Virginia: Technology and attribution needs for the FBI.

SESSION 7: Bioinformatics
Chairperson: J.P. Burans, U.S. Department of Homeland Security, Frederick, Maryland

O. White, Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore: Bioinformatics tools for genomics for identifying regions, genes, function.
T. Slezak, Lawrence Livermore National Laboratory, California: Bioinformatics tools and the BKC for attribution.

SESSION 8: Distinguishing Natural Outbreaks from Intentional Use
Chairperson: J. Smith, Federal Bureau of Investigation, Quantico, Virginia

P.S. Keim, Northern Arizona University, Flagstaff: Attribution using microbial databases.
S.A. Morse, Centers for Disease Control & Prevention, Atlanta, Georgia: Tracing disease outbreaks.
C.L. Cooke, National Counterproliferation Center, Washington, D.C.: Scenarios on interpretation of DNA evidence.
W.W. Laegreid, University of Illinois, Urbana: Response and interpretation: Finding the tainted mad cow.
D.L. Rock, University of Illinois at Urbana-Champaign: Interpreting FMDV outbreak data.

SESSION 9: Utility of Microbial Population Genetics and Legal Aspects
Chairperson: P.T. Pesenti, U.S. Department of Homeland Security, Washington, D.C.

D.E. Dykhuizen, Stony Brook University, New York: Tipping in phylogenetic analysis.
J. Yadav, University of Cincinnati College of Medicine, Ohio: Genomic approach for microbial pathogen detection and issues of interpretation.
R.P. Harmon, Alameda County District Attorney's Office, Oakland, California: HIV as a microbial forensic model.
T. Cebula, U.S. Food and Drug Administration, Laruel, Maryland: Summary of SWG mock trial: Impression of a day in court.

SESSION 10: Identification and Installation of Promising Technologies
Chairperson: A. Martinez-Fonts, Department of Homeland Security, Washington, D.C.

A. Martinez-Fonts, Department of Homeland Security, Washington, D.C.: Discussion on strategy for identifying promising technologies to support.

Wrap Up
Chairpersons: B. Budowle, Federal Bureau of Investigation, Quantico, Virginia; **S. Schutzer,** UMDNJ–New Jersey Medical School, Newark

Using Bar-code Data in Studies of Molecular and Evolutionary Dynamics

October 28–31

FUNDED BY **Alfred P. Sloan Foundation**

ARRANGED BY **D.E. Schindel,** Smithsonian Institution, Washington, D.C.
M. Blaxter, University of Edinburgh, United Kingdom
P. Gilna, University of California, San Diego
R.G. Harrison, Cornell University, Ithaca, New York
D.M. Rand, Brown University, Providence, Rhode Island
M. Veuille, Museum d'Histoire Naturelle, Paris, France

The Barcode of Life Initiative is generating an enormous volume of nucleotide sequence data from an homologous region of the animal mitochondrial genome. These data are being collected to build an information infrastructure for taxonomic research and for the rapid identification of species for diverse applied purposes such as border control of agricultural pests. This body of standardized gene sequence data may present allied fields of research with new and unanticipated opportunities as well. This workshop, the third in the series held at the Banbury Center, brought together population biologists, geneticists, bioinformaticians, and evolutionary biologists for the purpose of exploring the potential "off label" uses of DNA bar-code data.

Historical Background: J.H. Ausubel, The Rockefeller University, New York

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Overview of Bar-code Data
Chairperson: P. Gilna, University of California, San Diego

D.E. Schindel, Smithsonian Institution, Washington, D.C.: Linking bar-coded data to multiple users.
R. Hanner, University of Guelph, Ontario, Canada: Bar-code data standard and trace analysis.
S. Ratnasingham, University of Guelph, Ontario, Canada: The Barcode Life Data Systems: An informatics platform for the biodiversity informatics community.
M. Hajibabaei, University of Guelph, Ontario, Canada: Minimalist bar-code sequences.

SESSION 2: Species Boundaries, Speciation Processes, and Models
Chairperson: R.G. Harrison, Cornell University, Ithaca, New York

R.G. Harrison, Cornell University, Ithaca, New York: Multilocus approaches to defining species boundaries.
M. Blaxter, University of Edinburgh, United Kingdom: Defining and constructing MOTUs.
M. Stoeckle, The Rockefeller University, New York: Iterative taxonomy-DNA bar-coding cycle provides insights into species limits in birds.
J. Hey, Rutgers University, Piscataway, New Jersey: Population assignment likelihoods in a phylogenetic and demographic model.
A. Meyer, University of Konstanz, Germany: Sharing of mitochondrial DNA haplotypes in cichlid fishes.

SESSION 3: Phylogeography, Community Evolutions, and the Use of Bar Codes for Multispecies Studies
Chairperson: M. Veuille, Museum d'Histoire Naturelle, Paris, France

M. Veuille, Museum d'Histoire Naturelle, Paris, France: Can we extend intraspecific population genetics to community population genetics?
E. Bermingham, Smithsonian Tropical Research Institute, Balboa, Republic of Panama: Phylogeography of Caribbean birds.
G. Stone, University of Edinburgh, United Kingdom: Beyond the bar code: Setting our sites on reconstructing community evolution.
L. Knowles, University of Michigan, Ann Arbor: Statistical phylogeography.

SESSION 4: Selection on and Variation in Mitochondrial DNA Sequences
Chairperson: D.M. Rand, Brown University, Providence, Rhode Island

D.M. Rand, Brown University, Providence, Rhode Island: Bar codes and selection of mtDNA.
T. Barraclough, Imperial College London, Ascot, United Kingdom: Patterns of divergent selection from combined bar-code and phenotypic data.
R. Nielsen, University of Copenhagen, Denmark: Statistical approaches for DNA bar coding.
G. Wallis, University of Otago, Dunedin, New Zealand: Beyond the bar: Roles for a million COI sequences in studies of molecular adaptation.

SESSION 5: Visualization of Large Sequence Data Sets
Chairperson: D.E. Schindel, Smithsonian Institution, Washington, D.C.

M. Hajibabaei, University of Guelph, Ontario, Canada: Visualizing bar-code data.

SESSION 6: Final Discussion of Conclusions, Recommendation, and Action Items
Chairperson: D.E. Schindel, Smithsonian Institution, Washington, D.C.

E. Bermingham, M. Stoeckle

Interdisciplinary Memory Symposium in Neurosciences and the Humanities

October 31–November 2

FUNDED BY **The Selz Foundation, Inc.; The Satenik and Adom Ourian Educational Foundation; Haig R. Nalbantian; Marsh & McLennan Companies—MMC Matching Gifts to Education Program; The Daniel and Joanna S. Rose Fund, Inc.; Mr. and Mrs. Howard Phipps, Jr.**

ARRANGED BY **S. Nalbantian,** Long Island University, Brooklyn, New York
P. Matthews, GlaxoSmithKline, Oxford University, United Kingdom

The subject of human memory offers intriguing and exciting possibilities for interdisciplinary exchanges between the humanities and neuroscience. Just as the neuroscientist explores the physical workings of the brain with the tools of electrophysiology and molecular biology, so writers and artists explore and record the mental experiences of human beings. This interdisciplinary symposium discussed the ways in which the insights of those in the humanities can inform the models of human memory based on neuroscience.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

Introduction to the Symposium: S. Nalbantian, Long Island University, Brooklyn, New York; **Paul Matthews,** GlaxoSmithKline, Oxford University, United Kingdom

Keynote Address: Representations of Memory in Twentieth Century Art: Painting Modernism's Memories
L. Dalrymple Henderson, University of Texas, Austin

SESSION 1

J. Dubnau, Cold Spring Harbor Laboratory: The genetic basis of memory: Memories of a fly?
J.-P. Changeux, Institut Pasteur and Collège de France, Paris, France: Brain plasticity and the epigenetic variability of memory: Consequences in artistic contemplation and creation.
J.L. McClelland, Stanford University, California: Connectionist modeling of parallel processing and complementary memory systems.

SESSION 2

B. Favorini, University of Pittsburgh, Pennsylvania: The theatre of memory: The scene *is* memory!
S. Nalbantian, Long Island University, Brooklyn, New York: Literature as a laboratory for memory research: Literary case studies as evidentiary material.
J. Burt Foster, George Mason University, Fairfax, Virginia: Memory in the literary memoir: Nabokov, Yeats, Mary McCarthy.

SESSION 3: Interdisciplinary Panel for the Creation of a "Third Discourse" for Memory Research; Discussion
Chairpersons: S. Nalbantian, Long Island University, Brooklyn, New York; **P. Matthews,** Oxford University, United Kingdom

Topics for Discussion:

1. Identification of the most likely areas where humanists can illuminate the memory process in an exploratory or confirmatory fashion.
2. Pertinence of neuroscientific models to humanistic data.
3. Consideration of the way literature and the arts can be used as data with emphasis on artistic and linguistic factors. Possibilities for neuroimaging testing studies of human subjects.
4. Cross-disciplinary vocabulary.
5. Inroads to creativity.
6. Publication of a volume stemming from this symposium.

SESSION 4

F. Vidal, Max-Planck Institute for the History of Science, Berlin, Germany: The Cerebral subject: Memory, self and the brain in film.
Respondent: J.D. Talasek, National Academy of Sciences, Washington, D.C.
P. Matthews, GlaxoSmithKline, Oxford University, United Kingdom: Neuroimaging, memory, and brain disorders.
R. Stickgold, Harvard Medical School, Boston, Massachusetts: Dream analysis memory: Reactivation and reconsolidation.
Respondent: V. Doyère, CNRS, Université Paris Sud, Orsay, France

SESSION 5

D. Hertz, Indiana University, Bloomington: Poetry and music: What makes us remember?
M. Tramo, M.D., Harvard Medical School, Boston, Massachusetts: The neurobiology of memory for music.
P. Michon, Collège International de Philosophie, Paris, France: Epistemological models in social and neuroscientific memory studies: A philosophical inquiry.
Respondent: R. Phipps, Center for Process Studies, Claremont Graduate University, California: Whitehead's philosophy and memory.

J. Pierre-Changeux

From Statistics to Genes: Figuring Out the Molecular Basis of Complex Traits

November 4–7

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **A. Chakravarti,** The Johns Hopkins University School of Medicine, Baltimore, Maryland
L. Kruglyak, Princeton University, New Jersey

Studies of the genetic basis of complex traits are being transformed by a convergence of two related developments: genome-wide association studies that are providing a growing list of susceptibility loci for common diseases, and, studies of model organisms that are providing increasingly detailed descriptions at the molecular level. Both of these developments are being driven by new technologies, access to genomic sequence, and functional information. This meeting brought together leaders in three areas—human genetics, model systems, and technology—to chart the future course of understanding complex disease.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory
Overview: A. Chakravarti, The Johns Hopkins University School of Medicine, Baltimore, Maryland; **L. Kruglyak,** Princeton University, New Jersey

SESSION 1: Complex Traits in Model Systems I
Chairperson: L. Kruglyak, Princeton University, New Jersey

L. Steinmetz, EMBL Heidelberg, Germany: Fine mapping and functional characterization of complex traits in yeast.
G. Yvert, CNRS, Lyon, France: Cell-to-cell stochastic variation in gene expression as a complex trait in yeast.
J. McCusker, Duke University Medical Center, Durham, North Carolina: Complex traits in a simple eukaryote.
M. Nordborg, University of Southern California, Los Angeles: Whole-genome association in *Arabidopsis*.

SESSION 2: Complex Traits in Model Systems II
Chairperson: H.G. Parker, CGB/NHGRI/NIH, Bethesda, Maryland

M. Rockman, Princeton University, New Jersey: Complex trait genetics in *C. elegans*.
D. Stern, Princeton University, New Jersey: Morphological evolution through multiple *cis*-regulatory mutations at a single gene.
G.C. Gibson, North Carolina State University, Raleigh: *Drosophila* as a model for complex traits and diseases.
P. Wittkopp, University of Michigan, Ann Arbor: Genomic sources of regulatory variation.

G. Churchill, G. Yvert, M. Nordborg, A. Chakravarti

SESSION 3: Complex Trait Model Systems III
Chairperson: P. Wittkopp, University of Michigan, Ann Arbor

M. Shapiro, University of Utah, Salt Lake City: Genetic basis of parallel evolution in stickleback fish.
H.G. Parker, CGB/NHGRI/NIH, Bethesda, Maryland: The long and short of canine fixed trait mapping.
B.A. Hamilton, University of California, San Diego; School of Medicine, La Jolla, California: Architectures of modifier gene networks: Thoughts from two examples.
G.A. Churchill, The Jackson Laboratory, Bar Harbor, Maine

SESSION 4: Human Complex Trait Dissection and Evolution
Chairperson: L. Pennacchio, Lawrence Berkeley National Laboratory, California

D.B. Goldstein, Duke University, Durham, North Carolina: Genome-wide association studies in host response to HIV.
A.C. Kong, DeCode Genetics, Reykjavik, Iceland: Recent gene discoveries and new challenges.
M. McCarthy, The Churchill Hospital, Oxford, United Kingdom: Thinking big: Genes involved in type-2 diabetes, adiposity, and height.
S. Deutsch, University of Geneva Medical School, Switzerland: Phenotype mapping in cell lines.
S. Tishkoff, University of Maryland, College Park: Genetic and phenotypic variation in Africa.
G. Wray, Duke University, Durham, North Carolina: Genome-wide imprints of selection and the evolution of complex traits in humans.

SESSION 5: New Technologies
Chairperson: A. Chakravarti, The Johns Hopkins University School of Medicine, Baltimore, Maryland

S. Kruglyak, Illumina, Inc., San Diego, California: Tools for whole-genome association studies.
J. Sebat, Cold Spring Harbor Laboratory: Analysis of genome copy-number variation in psychiatric disease.
L. Pennacchio, Lawrence Berkeley National Laboratory, California: Deep resequencing in the Dallas Heart Study.
T.S. Mikkelsen, Broad Institute, Cambridge, Massachusetts: Mammalian epigenomics.

SESSION 6: Final Discussion and Future Directions

Discussion
A. Chakravarti, The Johns Hopkins University School of Medicine, Baltimore, Maryland, and L. Kruglyak, Princeton University, New Jersey.

Second Environment Ontology Workshop

November 14–16

FUNDED BY **U.S. National Science Foundation's Research Coordination Network grant (DBI 0234147) to the Gramene Database**

ARRANGED BY **P. Jaiswal,** Cornell University, Ithaca, New York
N. Morrison, University of Manchester, United Kingdom
D. Field, Oxford University, United Kingdom
S. Lewis, Lawrence Berkeley National Laboratory, California
B. Smith, University at Buffalo, New York
M. Ashburner, University of Cambridge, United Kingdom

An ontology is a controlled, structured vocabulary developed to represent entities in a given domain and the relations between them. The use of a standardized, consistent nomenclature means that information can be searched by computers, enabling them to share and integrate information without human intervention. There is, for example, a Gene Ontology that has been developed so that different genome databases can "talk" to each other. Participants in this workshop are attempting do derive an ontology to describe the environments in which organisms live. Such an ontology would facilitate the retrieval of any biological record anchored to the environment ontology, whether in sequence or genome databases, tissue banks, or museum collections. Developing (and implementing) such ontologies is a far from trivial endeavor and participants learned how Gene Ontology developed as well as planning future steps.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1
Chairperson: P. Jaiswal, Cornell University, Ithaca, New York

P. Jaiswal, Cornell University, Ithaca, New York: Welcome and introductory remarks.
B. Smith, University at Buffalo, New York: EnvO introduction.
M. Ashburner, University of Cambridge, United Kingdom: Progress on EnvO+GAZ ontology development.
N. Morrison, University of Manchester, United Kingdom: Ontology maintenance and Web site + database logistics.
C. Mungall, Lawrence Berkeley National Laboratory, California: Phenote annotation tool.

Case Studies with EnvO annotations:
P. Dawyndt/Bart van Brabant
L. Schriml/Aaron Gussman
D. Field
N. Sarkar

SESSION 2
Chairperson: N. Morrison, University of Manchester, United Kingdom

New Case Studies for Potential EnvO Annotations
J. White
S. Greene
L. Hirschman
V. Markowitz
S. Ratnasingham
M. Ashburner, University of Cambridge, United Kingdom: Hands-on work on ontology.

SESSION 3
Chairperson: B. Smith, University of Buffalo, New York

S. Lewis, University of California, Berkeley: Summary from Day-1.
M. Ashburner, University of Cambridge, United Kingdom: Hands-on work on ontology.

SESSION 4
Chairperson: S. Lewis, University of California, Berkeley

M. Ashburner, University of Cambridge, United Kingdom: Hands-on work on ontology.
B. Smith, University of Buffalo, New York: Discussion of funding possibilities.
N. Morrison, University of Manchester, United Kingdom: Identify potential projects, databases, and collaborators.
S. Lewis, University of California, Berkeley: Summarizing the meeting, action items, and wrap up.

L. Hirschman

Podosomes and Invadopodia: Signatures of the Wandering Cell?

November 26–29

FUNDED BY **Cold Spring Harbor Laboratory Corporate Sponsor Program**

ARRANGED BY **G. Jones,** King's College, University of London, United Kingdom
J.S. Condeelis, Albert Einstein College of Medicine, Bronx, New York
A. Huttenlocher, University of Wisconsin, Madison

Examples of cell migration include leukocytes involved in immune surveillance and innate immunity, and other cell types during morphogenetic movements of embryonic development, in wound healing, and in the invasion and dispersal of metastatic tumor cells. To a large extent, these cell movements depend on the degradation of extracellular matrix components by focal secretion of matrix metalloproteinases. Localized degradation of the matrix is found at adhesive (podosomes) and protrusive (invadopodia) locations in a variety of cell types including leukocytes and invasive carcinoma cells. Research on the role of these transient membrane-associated organelles in cell motility suggests that they are involved in directed cell migration and chemotaxis in vitro and in vivo. Participants reviewed recent findings and developments and discussed the importance of these structures in cell migration, inflammation, morphogenesis, and metastasis. There was vigorous discussion of the relationship between podosomes and invadopodia.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Principles of Cell Motility
Chairperson: G. Jones, King's College London, United Kingdom

General Discussion on Terminology: Do podosomes = invadopodia = invadosomes?

Discussion Leaders:
G. Jones, King's College, University of London, United Kingdom
J.S. Condeelis, Albert Einstein College of Medicine, Bronx, New York

A. Huttenlocher, University of Wisconsin, Madison: Principles of cell motility: Neutrophil chemotaxis in vivo.

P. Friedl, University of Würzburg, Germany: Invadopods in three-dimensional invasion?

J.S. Condeelis, Albert Einstein College of Medicine, Bronx, New York: Invadopod regulation and function in breast tumor metastasis.

F. Gertler, Massachusetts Institute of Technology, Cambridge:

An invasion-specific Mena isoform promotes cancer cell invasion and potentiates EGF responses.
S. Muthuswamy, Cold Spring Harbor Laboratory: Changes in cell polarity pathways regulate cell invasion.
P. DeCamilli, Yale University, New Haven, Connecticut: Role of dynamin in cell physiology.

SESSION 2: Podosome Regulation
Chairperson: A. Huttenlocher, University of Wisconsin, Madison

G. Jones, King's College London, United Kingdom: Podosomes in myeloid leukocytes.
S. Linder, University of Munich, Germany: Regulation of podosome dynamics in human macrophages.
R. Buccione, Consorzio Mario Negri Sud, Italy: Regulation of invadopodia biogenesis.
D. Cox, Albert Einstein College of Medicine, New York: WASP phosphorylation and podosome regulation.
R. Baron, Yale University, New Haven, Connecticut: Src regulation of podosomes using the osteoclast as a model system.
I.M. Anton, Centro Nacional de Biotecnología/CSIC, Spain: Contribution of WIP to podosome formation in dendritic cells and mature osteoclasts.
S. Tsuboi, Burnham Institute for Medical Research, La Jolla, California: Role of the WASP-WIP complex in podosome formation in macrophages.

SESSION 3: Actin-based Motility
Chairperson: J.S. Condeelis, Albert Einstein College of Medicine, Bronx, New York

L.M. Machesky, Cancer Research UK, United Kingdom: Role of Arp2/3 complex and IRSp53-MIM proteins in actin membrane interactions.
J. Taunton, University of California, San Francisco: Actin-dependent feedback to N-WASP: A signal amplification mechanism for invadopodia expansion.
J.A. Cooper, Washington University, St. Louis, Missouri: Role of cortactin and HS1 in actin assembly in osteoclasts and lymphocytes.
J.E. Bear, University of North Carolina, Chapel Hill: Role of coronins in cancer cell invasion and motility.
J. Theriot, Stanford University, California: Large-scale coordination of actin polymerization, contraction, and adhesion in motile keratocytes.

SESSION 4: Cell Signaling in Invasive Motility
Chairperson: F. Gertler, Massachusetts Institute of Technology, Cambridge

S.A. Courtneidge, Burnham Institute for Medical Research, La Jolla, California: Role of the adaptor protein Tks5 in cancer cell invasion and embryonic development.
A.S. Mak, Queen's University, Kingston, Canada: Calpain-4 in podosome formation.
M.A. Chellaiah, University of Maryland, Baltimore: Podosomes and invadopodia: Role in extracellular matrix degradation and migration in osteoclasts and prostate cancer cells.
E. Genot, Université Bordeaux, Pessac, France: Endothelial podosomes: A new tool for remodeling the vascular bed.
H. Gil-Henn, Yale University School of Medicine, New Haven, Connecticut: Pyk2 in podosome organization and bone remodeling.
S.C. Mueller, Georgetown University Medical Center, Washington, D.C.: Invadopodia: Src and the cell-protease interface.
S.A. Weed, West Virginia University, Morgantown: Phosphoregulation of cortactin function.

SESSION 5: Invadopodia and Cancer Invasion
Chairperson: J.S. Condeelis, Albert Einstein College of Medicine, Bronx, New York

C. Streuli, University of Manchester, United Kingdom: Integrins in breast development and cancer.
E. Sahai, Cancer Research UK, London, United Kingdom: Mechanisms of cell invasion in three-dimensional environments and in living tumors.
A. Weaver, Vanderbilt University Medical Center, Nashville, Tennessee: Microenvironmental regulation of invadopodia.
J.F. Marshall, University of London, United Kingdom: Targeting integrin α-v β-6 for the imaging and therapy of carcinoma.
N. Carragher, AstraZeneca, Loughborough, United Kingdom: Modeling distinct modes of tumor cell invasion for drug discovery.

General Discussion and Terminology: Do podosomes = invadopodia in composition and function?
Discussion Leaders:
A. Huttenlocher, University of Wisconsin, Madison
G. Jones, King's College London, United Kingdom
J.S. Condeelis, Albert Einstein College of Medicine, Bronx, New York


S. Courtneidge, G. Jones


S. Mueller, R. Buccione

Cell Transplantation as a Therapy for Parkinson's Disease

December 9–12

FUNDED BY **Private support**

ARRANGED BY **A. Bjorklund,** University of Lund, Sweden
H. Cline, Cold Spring Harbor Laboratory
O. Lindvall, Lund University Hospital, Sweden

Previous clinical trials have shown that transplants of fetal dopamine neurons can survive and function in the brains of patients with Parkinson's disease. Therapeutically valuable and long-lasting clinical improvement has been observed in subgroups of patients, but troublesome graft-induced dyskinesias have been noted in a significant number of cases. Further development of a dopamine cell replacement therapy for Parkinson's disease will critically depend on the development of alternative sources of cells for grafting. Participants critically examined the issues that need to be resolved in order to bring the cell replacement approach into a clinically successful and competitive therapy for patients with Parkinson's disease.

Introductory Remarks: J.A. Witkowski, Banbury Center, Cold Spring Harbor Laboratory

SESSION 1: Clinical Trials: A Critical Assessment of the Results Obtained in Patients with Fetal DA Neuron Transplants
Chairperson: D. Eidelberg, Feinstein Institute for Medical Research, Manhasset, New York

T.B. Freeman, University of South Florida, Tampa: The Tampa–Mount Sinai trial.
C.R. Freed, University of Colorado School of Medicine, Denver: The Denver–Columbia trial.
I. Mendez, Dalhousie University, New York: The Halifax program.
O. Lindvall, Lund University Hospital, Sweden: The Lund–London–Marburg program.
T.B. Freeman, University of South Florida, Tampa: Neurosurgical aspects.

SESSION 2: What Is Required to Make a Cell Transplantation Therapy Clinically Competitive?
Chairperson: R.A. Barker, University of Cambridge, United Kingdom

G. Nikkhah, Abt. Stereotaktische Neurochirugie, Freiburg, Germany: Pattern and extent of recovery in grafted patients.
S. Dunnett, Cardiff University, South Wales, United Kingdom: Behavioral criteria in rodent models.
D.E. Redmond, Yale University School of Medicine, New Haven, Connecticut: Behavioral criteria in primate models.
A. Bjorklund, University of Lund, Sweden: Necessary properties of grafted cells.

SESSION 3: Generation of Dopamine Neurons from ES Cells
Chairperson: S. Dunnett, Cardiff University, South Wales, United Kingdom

B. Reubinoff, Hadassah University Medical Organization, Jerusalem, Israel: Human embryonic stem cells for Parkinson's disease.
J. Ericson, Karolinska Institute, Stockholm, Sweden: Intrinsic determinants in stem cell engineering.
K.-S. Kim, McLean Hospital, Harvard Medical School, Belmont, Massachusetts: Potential cell sources and animal models for Parkinson's disease.
E. Arenas, Karolinska Institute, Stockholm, Sweden: Reduced proliferation and enhanced dopaminergic differentiation by Wnt5a.

SESSION 4: In Vivo Performance of Stem-cell-derived Dopamine Neurons: Survival, Function, Tumorigenesis
Chairperson: A. Bjorklund, University of Lund, Sweden

L. Studer, Memorial Sloan-Kettering Cancer Center, New York: Directed differentiation and purification of human ES-cell-derived dopamine neurons.
S.-C. Zhang, University of Wisconsin, Madison: Survival and function of DA neurons derived from hES cells.
S.A. Goldman, University of Rochester Medical Center, New York: DA neurons derived from human ES cells in coculture.
J. Takahashi, Kyoto University, Japan: In vivo functional studies in primates.

SESSION 5: Critical Issues for the Development of a Stem Cell Therapy for Parkinson's Disease
Chairperson: O. Lindvall, Lund University Hospital, Sweden

Theme 1: Transplant-induced Dyskinesia

Speaker: K. Steece-Collier, University of Cincinnati, Ohio
Discussant: D. Kirik, University of Lund, Sweden
Open Discussion

Theme 2: Strategies to Avoid Tumor Formation

Speaker: V. Tabar, Memorial Sloan-Kettering Institute for Cancer, New York
Discussant: J. Takahashi, Kyoto University, Japan
Open Discussion

Theme 3: Patient Selection and Efficacy

Speaker: R.A. Barker, University of Cambridge, United Kingdom
Discussants: D. Eidelberg, Feinstein Institute for Medical Research, Manhasset, New York; **P. Piccini,** Imperial College London, United Kingdom
Open Discussion

P. Piccini

V. Tabar, S. Goldman

DOLAN DNA LEARNING CENTER

DOLAN DNA LEARNING CENTER EXECUTIVE DIRECTOR'S REPORT

Preparing students and families to thrive in the gene age

ADMINISTRATION	INSTRUCTION	BIOMEDIA	TECHNOLOGY DEVELOPMENT
Mary Lamont	**Elna Carrasco**	**Steven Blue**	**Cornel Ghiban**
Valerie Meszaros	**Jennifer Cutillo**	**John Connolly**	**Bruce Nash**
David Micklos	**Natalia Hanson**	**Eun-Sook Jeong**	**Jermel Watkins**
Karen Orzel	**Brian Lang**	**Susan Lauter**	
Carolyn Reid	**Erin McKechnie**	**Chun-hua Yang**	
	Amanda McBrien		
	Lauren Weidler		

For practical, philosophical, and political reasons, plant research has lagged behind medically oriented research. Practically, the plant cell presents its own tremendous barrier to research. Although animal cells are surrounded by a single permeable membrane, plant cells also include a tough cell wall. This "wooden box" makes plant cells difficult to genetically engineer. Transformation—the process of inserting a new DNA sequence into a living cell and the constraining point in the genetic engineering of a species—was first accomplished in bacteria in the 1970s and in animal cells in the 1980s. However, plant cells could not be reliably transformed until the 1990s.

Philosophically, humans are naturally self-absorbed with improving the quality and length of their own lives. After World War II, this desire was translated into political will by Vannevar Bush. As director for the Office of Scientific Research and Development (OSRD), he had marshaled the academic research effort in support of the war. In his 1945 report, *Science, The Endless Frontier*, Bush eloquently argued that the federal government should maintain the strong sponsorship of academic research it had established during the war, but to bend it toward fostering improved public health. This led to the rapid expansion of the National Institutes of Health (NIH) and its rise as the preeminent medical research organization in the world. In the 1960s, Mary Lasker joined with Senators J. Lister Hill and John E. Fogarty to raise public and political awareness of cancer, which stimulated the ascension of the National Cancer Institute as the largest of all the national institutes.

Plant research had received early federal support through the system of land grant universities established by Congress in 1862. Now sponsored by the Department of Agriculture, this decentralized research program produced the "green revolution" that dramatically increased crop yields through a combination of improved plant cultivars, petrochemical fertilizers, and mechanization. However, despite the lobbying of congressmen from agricultural states, in the post-war era, the Department of Agriculture's research budget fell far behind NIH's. At $2.3 billion, the Department of Agriculture's 2007 research budget was less than 10% of the NIH's $28.4 billion budget.

In the last 10 years, basic plant research has received a new champion—the National Science Foundation (NSF). Given NIH's public health mandate, NSF has been excluded from funding most human or medically oriented research. Thus, NSF sat on the sidelines during the sequencing of the human genome and important model organisms. However, with the advent of the National Plant Genome Initiative (NPGI) in 1988, NSF rose to fill a vacuum in plant genome sequencing that was not being filled by the Department of Agriculture. NSF became the lead agency in the focused effort to determine the DNA sequences of the grains and other crop plants that feed most of the world's population—with the hope that insights into plant genomes would fuel a second green revolution, allowing us to feed the ten billion people expected to occupy the planet by 2050. During the last decade, NPGI provided $780 million in research funding, culminating in a $29.5 million NSF project to sequence the maize (corn) genome.

iPlant Consortium

With the maize genome sequence imminent, NSF announced in 2007 a $50 million grant competition to establish a cyberinfrastructure to integrate genome data with insights across many disciplines of plant research. The DNALC was fortunate to have been included in the *iPlant Consortium*, headed by the University of Arizona, which won this 5-year award. Through a series of symposia and workshops, the *iPlant Consortium* will engage the research community to identify a number of "grand challenges" whose resolution will include input from scientists working at all levels of plant function and organization: molecular biology, genetics, genomics, biochemistry, cell biology, physiology, plant breeding, systematics, ecology, and evolutionary biology. Each *Grand Challenge* Team will then work with computer scientists at the University of Arizona and Cold Spring Harbor Laboratory to develop a "discovery environment" that will provide bioinformatics and database tools to integrate findings across disciplines. As a member of the Education, Outreach, and Training (EOT) Component, the DNALC will work with the project team to embed outreach materials within the *iPlant* portal, thus tightly linking plant research and education.

The DNALC will receive $2.1 million to develop an educational gateway at the *iPlant* portal and work with the *Grand Challenge* Teams to develop educational interfaces to the *Discovery Environments*. Our objective is to get tools and data sets into the hands of high school and college faculty who are "gatekeepers" at the cusp of research and science education. We will extract elements from the *Discovery Environments* and package them with example data in intuitive, visually appealing interfaces. The objective is to engage novices and allow them to quickly learn the rudiments of integrative analysis and then generalize these skills to gain insight into biological concepts. Whenever possible, each tool will be packaged in an attractive "skin" and take on the properties of a stand-alone, desktop object.

During years 2–5 of the grant, we will conduct a nationwide program of 1.5-day workshops to train 1000 science teachers on how to use the *iPlant* tools for student projects that support integrative and computational thinking. The workshops will target faculty in the "2+2+2" continuum of advanced high school, two-year college, and four-year college. These biologists will need a basic understanding of how to use the *Discovery Environments* and educational interfaces to apply to their own research and to use with classes they teach.

Continuing Plant Research Collaborations

Plant biologists are now in transition from an era when their experiments were limited by the time required to collect new data to a new age when there is literally too much new data to effectively analyze. Faced with exponentially accumulating data from genome sequencing projects, plant scientists rely heavily on computers to search for genes and other functional components of chromosomes. The *iPlant* award marked the culmination of the DNALC's continuing effort to train teachers on how to access this sort of genome data and to analyze it with the same tools used by research biologists.

In 2007 we thus continued several collaborations with researchers to provide high school and college faculty access to the new world of plant genomics. We worked with CSHL researchers Lincoln Stein and Doreen Ware on their NSF-funded *Gramene* project, which provides informatics tools for analyzing and comparing grain genomes. Our companion Internet site, *Dynamic Gene* (www.dynamicgene.org), provides background information on gene analysis and bioinformatics tools that enable students to analyze genes from the newly sequenced rice genome. Because many of these genes have only been predicted using computer algorithms, students may well be the first "scientists" to examine many of these genes in detail.

Animated tutorials in the *Meaning, Structure*, and *Evidence* sections of the site explain how DNA sequence encodes information, how computers identify patterns that predict gene structures, and how experimental evidence complements computer predictions to correctly identify genes. The *Annotation* section provides detailed instructions on how to analyze a predicted gene with *Apollo*, research software developed to analyze the *Drosophila* genome. The *Projects* section allows students to download fragments of the rice genome, annotate predicted genes, and upload their results to compare with classmates or share with researchers. During the year, Faculty Fellows Bob Wheeler (Pine Creek High School, Col-

orado Springs, Colorado) and Debra Burhans (Canisius College, Buffalo, New York) contributed many improvements to the site, including narrated tutorials and animations on experimental evidence used to confirm computer predictions.

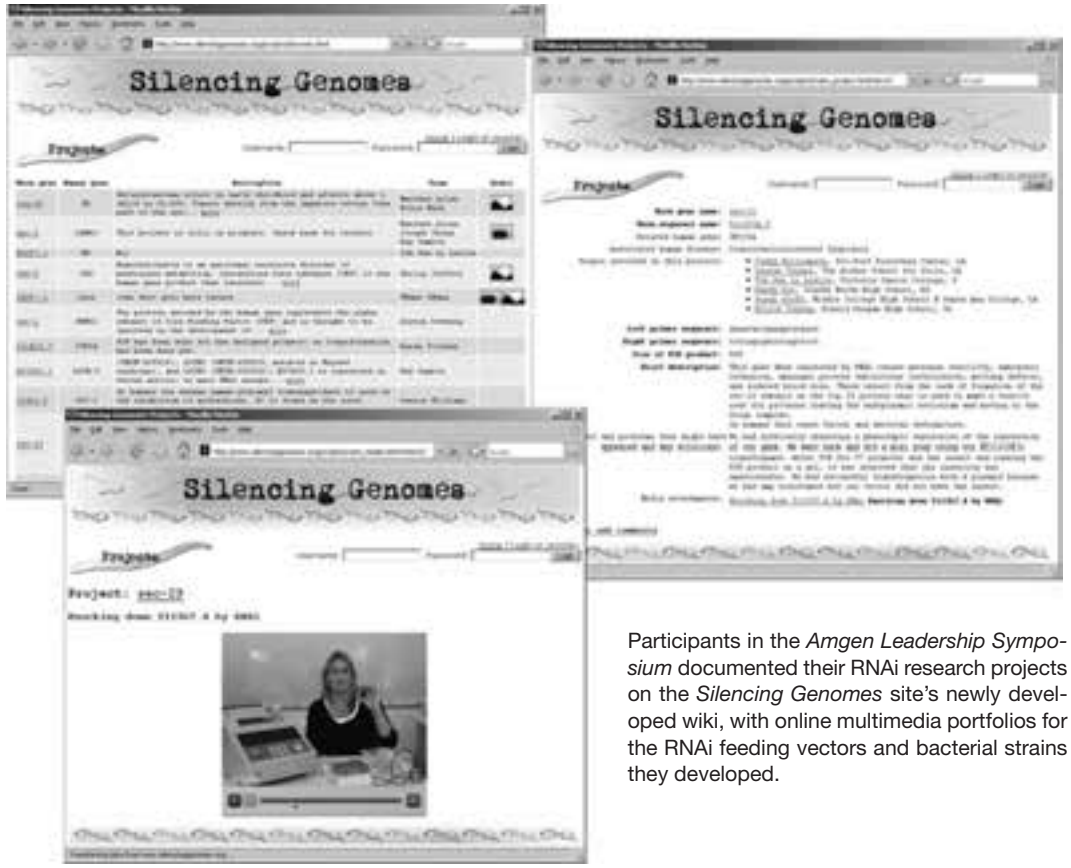
We continued our collaboration with CSHL researchers Dick McCombie and Marja Timmermans, and Mike Scanlon at Cornell University to encourage underrepresented minorities to teach and study plant genomics. In past summers, six Faculty Fellows have spent 2 weeks working in plant genome labs at CSHL or Cornell and 1 week working with us at the DNALC. In 2007, we collaborated with Faculty Fellows Gokhan Hacisalihoglu and Diomede Buzingo to conduct teacher training at their home institutions—Florida Agricultural & Mechanical University (Tallahassee) and Langston University (Oklahoma). These 3-day workshops presented a lab and Internet-based curriculum on modern plant research to mixed groups of high school and college faculty. Fellows Olga Kopp, Mary Smith, Muhammad Mian, and Javier Gonzalez also hosted 1.5-day follow-up workshops at their institutions: Utah Valley State College (Orem, Utah), North Carolina Agricultural & Technical State University (Greensboro, North Carolina), Rust College (Holly Springs, Mississippi), and Texas Agricultural & Mechanical University (Weslaco, Texas). Building on previous workshops conducted at each site, the follow-ups introduced teachers to mobile "footlockers" that include PCR (polymerase chain reaction) and electrophoresis equipment needed to conduct experiments that test for GM foods and to analyze transposon polymorphisms in maize and *Arabidopsis*. Workshop participants can borrow the equipment footlockers from host institutions for use in their classes, removing a major barrier to the implementation of modern experiments in plant molecular genetics.

Record Year for New Grants

The educational grant programs to which the DNALC applies are suffering from the same low funding rates as research programs. Evidence of the dire straights at the NSF: There are no longer any distinct programs to provide in-service training for science teachers. These programs had, since the 1950s, provided summer institutes that kept science teachers up to date and were a major source of funding for our own training activities. Despite this extremely difficult funding situation, we obtained three new teacher-training grants. Along with the *iPlant* award, these grants brought to $3.67 million the total of new federal and foundation grants received—our largest 1-year total since opening in 1988.

NSF Course, Curriculum, and Laboratory Improvement Program: Phase II Project ($444,134 over 2 years). Today's biology students will face the future challenge of unraveling the biological meaning of the millions of genome sequences that are rapidly accumulating in DNA databases. RNA interference (RNAi) provides a powerful tool to move directly from DNA sequence to the analysis of gene function in living organisms. During Phase I of this project, we developed an experiment- and bioinformatics-based curriculum, *Silencing Genomes*, that explores RNAi in the model eukaryotic organism, *Caenorhabditis elegans*. The curriculum begins with observation of mutant phenotypes and basic worm "husbandry" and then progresses to simple methods to induce RNAi and use RNAi to rescue (compensate) a mutant phenotype. A more advanced experiment uses "single-worm PCR" to examine the mechanism of RNAi, comparing the DNA of worms with identical phenotypes induced by either RNAi or a gene mutation. The curriculum culminates with open-ended methods that support student projects. Students can perform RNAi "from scratch" using bioinformatics to identify a target gene and develop their own RNAi reagents. Students also have free access to the DNALC's collection of RNAi feeding strains, which can be used to screen for genes involved in a particular biological pathway.

An online lab notebook *Silencing Genomes* (www.silencinggenomes.org) combines lab methods with user-friendly features adapted from the DNALC's popular text *DNA Science*, including flow charts, reagent recipes, and extensive instructor information. Supporting resources include photos and video of *Caenorhabditis elegans* mutants, as well as a simple checkout system to obtain any of 80 *C. elegans* mutants and *Escherichia coli* feeding strains. The site also provides a launch pad for bioinformatics exercises that accompany each experiment. This is, to our knowledge, the first integrated set of RNAi experiments specifically designed for the teaching laboratory, and the completion of the Phase I project coincided with the awarding of a 2006 Nobel Prize for the discovery of RNAi.



Participants in the *Amgen Leadership Symposium* documented their RNAi research projects on the *Silencing Genomes* site's newly developed wiki, with online multimedia portfolios for the RNAi feeding vectors and bacterial strains they developed.

The Phase II project will introduce the *Silencing Genomes* curriculum to 208 college teachers at week-long workshops conducted at eight sites nationwide. At least half of the workshops will be conducted at historically Black or Hispanic institutions, which will triple participation by underrepresented minorities. In a unique capacity-building effort, workshop participants will collaborate to develop targeting vectors to silence approximately 100 *C. elegans* genes, which will be freely available to students and teachers. During a 1.5-day follow-up workshop, participants will test their feeding vectors and develop online multimedia portfolios, including videos, a notes-enabled *Wiki* report, and a simple bulletin board. Thus, each vector will include its own evolving record of how it has been developed and used by a community of committed "experts."

NIH Science Education Partnership Award: Phase II Project ($377,644 over 2 years). With Phase I funding, we developed *Inside Cancer* (www.insidecancer.org), a multimedia Internet resource for understanding the molecular genetic basis of cancer. On a biological level, the site takes students inside the cell to explore the molecular and genetic roots of cancer. On a sociological level, it provides the insider's perspective of the world of cancer research. One objective is to help students understand how modern concepts from molecular and cellular genetics are being integrated into ideas about cancer diagnosis, prevention, and treatment. Another objective is to allow students to learn modern biology in the same way as basic cancer researchers—by meeting scientists, seeing how experiments are done, and visualizing the unseen world of genes and molecules.

The Phase II project will focus on disseminating the *Inside Cancer* site to precollege teachers and evaluating its educational effects. During the term of the grant, 800 secondary biology and health teachers will receive intensive training at 1-day workshops held at 20 sites nationwide. The workshops will be

conducted in conjunction with teacher professional meetings and science outreach programs at universities and historically Black and Hispanic institutions. Forty workshop participants will receive fellowships to conduct second-round training to reach an additional 640 teachers. Participants' teaching behaviors will be monitored over time, and a controlled study will compare attitudinal and learning effects among 280 high school students. We will also develop an online *Teacher Center* with links to teaching standards, tools to build custom multimedia presentations, and an exchange for collaboratively generated lesson plans.

Howard Hughes Medical Institute (HHMI) Initiative for Biomedical Research Institutions ($746,243 over 5 years). This professional development program is a collaboration with the New York City Department of Education (DOE) to develop a strong base of teachers who can competently introduce six "targeted" experiments in genetics and biotechnology at identified points in required science courses. The program focuses on the 8th and 9th grade levels, where all students receive their first, and often last, exposure to genetics and biotechnology. During the course of the grant, 820 teachers will receive 4 days of training, which will certify them to implement the target labs. Embedded within the school year, the certificate training will allow educators to readily implement their new knowledge and abilities. Two weeks of leadership training conducted during the summer will then allow 160 teachers to extend their expertise, develop student research capabilities, and formulate biology electives. An equipment footlocker and reagent kit administered by the DNALC will also support each targeted lab.

The project will be unique in the extent to which student and purpose-built computer tools and multimedia items will support teacher learning. A mini-Internet site, or *Lab Center*, will accompany each targeted laboratory and will include (1) a video introduction to the experiment, (2) interactive and PDF versions of the experiment, (3) follow-up activities, (4) science stories that relate the experiment to local research, (5) scientist interviews, (6) animations, and (7) selected links. Registered teachers will be able to customize the *Lab Center* to include class name, selected links, bulletin board for class announcements, and lab results. An editor will allow teachers to select from more than 3000 multimedia items and include them in windows within the *Lab Center*. Each customized *Lab Center* will be stored on the DNALC server and accessed by students using a unique address associated with the teacher's profile. We will increase the local appropriateness of instruction by linking each lab to NY "science stories," a series of minidocumentaries about the past or current work of notable researchers from CSHL, The Rockefeller University, Columbia University, American Museum of Natural History, Cornell University, and other New York institutions.

Harlem DNA Lab

The HHMI was a substantial step toward the fruition of our dream of a DNALC facility to serve the students and teachers of New York City. Since its founding in 1988, the DNALC has provided hands-on experiments for a third of a million students from the New York Metropolitan area during half-day field trips, in-school instruction, and week-long DNA camps. The natural clientele for this enrichment has been students who attend schools within a 40-minute commute of Cold Spring Harbor. In 2002, we partnered with the North Shore–Long Island Jewish Health System to open a satellite facility, DNALC *West*, in Lake Success. However, because of rules that restrict New York City school buses from traveling outside the city, the DNALC's local programs have primarily benefited the relatively affluent school systems of Nassau and Suffolk Counties.

In July 2006, we received a $50,000 planning grant from the Goldman Sachs Foundation to develop support for a satellite center to extend the DNALC's expertise in genetics instruction to the students and teachers of New York City. As part of the HHMI proposal, New York City Schools Chancellor Joel Klein provided us exclusive use of a 1200-square-foot classroom in the John S. Roberts Educational Complex (MS45) in East Harlem in which to provide teacher training. By year's end, this space had been renovated identically to our facility in Cold Spring Harbor, including our signature student lab desks, and opened on schedule in early spring 2008.

Because the HHMI teacher-training program will occupy only about 20% of the lab's capacity, the facility will support a broader program of science enrichment to New York City students. *Harlem DNA Lab*

Renovations to the former graphics lab in J.S. Roberts Junior High School were near completion in early December.

will serve approximately 4000 6th to 12th grade students per year, offering them access to accelerated lab experiences that heretofore have been primarily offered to students from Long Island. The Jerome L. Greene Foundation provided $100,000 for equipment for experiments ranging from basic genetics and cell microscopy to modern DNA manipulation and forensic biology. State-of-the art gene chip readers and DNA sequencers will help us begin to fulfill our vision of enabling every New York City student to see their own DNA before they graduate from high school.

During the academic year, each John S. Roberts student will participate in three to four genetics laboratories at the facility, and students from schools throughout New York City will visit the facility for half-day "lab field trips." During the summer, week-long camps will provide intensive enrichment for top students citywide. (The DNALC has applied for funding from the New York State Excelsior Scholars Program to support 4 weeks of instruction for middle school students in summer 2008.) A cadre of student interns will provide lab support and conduct independent research projects that will allow them to compete in state and national science competitions—and seamlessly transition to research projects at local universities and research institutions.

All activities are sequenced, so that a student can grow academically and be tracked through multiple experiences over a period of years. The experiments embody key concepts and process skills of the New York City *Scope and Sequence for Science*, the New York State *Science Core Curriculum*, and the

Harlem DNA Lab

The Harlem DNA Lab logo is used on brochures, letterhead, and the *Harlem DNA Lab* Internet site (www.dnalc.org/harlemdnalab/).

National Science Education Standards. The lab program is also in synch with New York City's strong existing base of biological and clinical research and the emerging biotechnology industry that will be supported at the new East River Science Park.

Harlem DNA Lab will make cost-effective use of instructional technology and methods developed with more than $26 million in federal and foundation grants. The venture will also draw on our experience in establishing teaching centers worldwide. Like DNALC *West*, the *Harlem DNA Lab* will be a satellite—directly administered by the DNALC and staffed by DNALC personnel. In this way, we can control the quality of instruction and offer New York City students an experience that is identical to that received by their Long Island peers.

Harlem DNA Lab can provide a reproducible model for how science institutions can interact with large school systems to help transform science education for urban students. We also hope that it will establish our presence in Manhattan and provide a springboard for the establishment of a stand-alone facility equal in size to the Dolan DNALC (10,000 square feet).

Supporting Real Forensic Biology

In the mid-1980s, British researcher Sir Alec Jeffreys coined the term "DNA fingerprinting" and was the first to use DNA variations (polymorphisms) in human paternity, immigration, and murder cases. Jeffreys discovered a class of repeat polymorphisms, termed VNTRs (variable number of tandem repeats), in which a DNA sequence is repeated end-on-end. Like boxcars in a train, different numbers of DNA repeats create alleles that differ in length. The alleles inherited from mother and father can be separated by electrophoresis, and the specific combination is "scored" as a genotype.

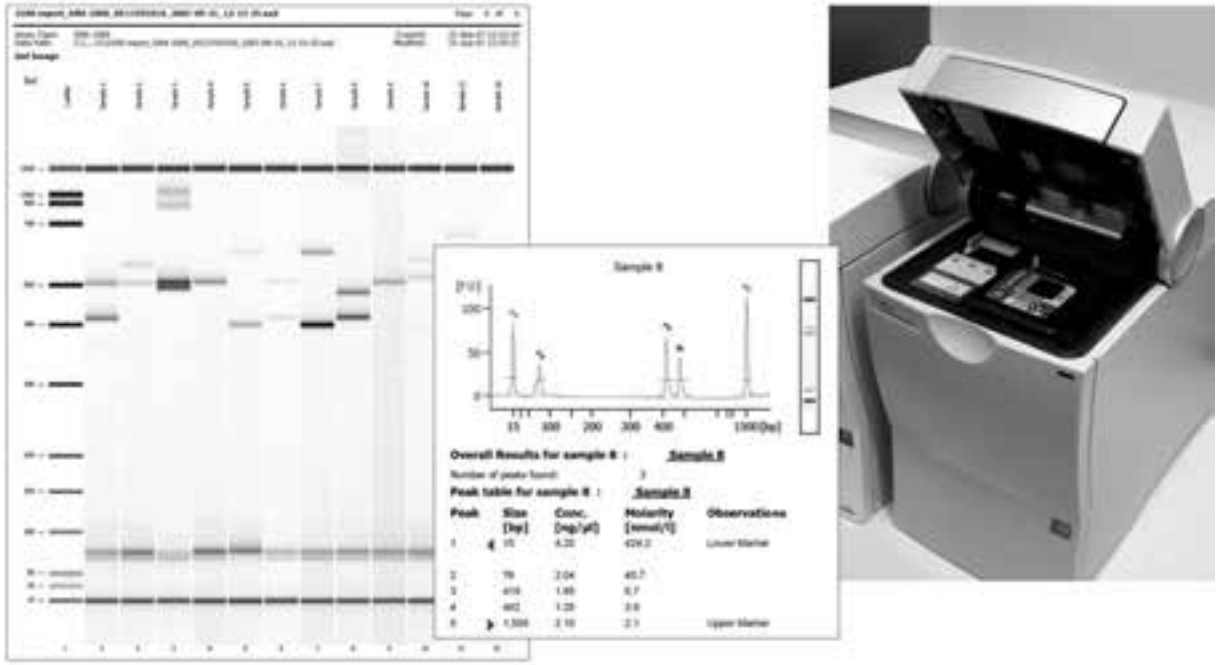
In the late 1980s, the DNALC worked in parallel to researchers as they developed a PCR-based assay for human VNTRs called D1S80. Although D1S80 became the first amplified polymorphism used in forensic biology—and was part of the evidence in the O.J. Simpson murder case—the DNALC abandoned this experiment because the different alleles proved to be impossible to distinguish using simple gel electrophoresis. In the meantime, human forensic analysis moved on to STRs (short tandem repeats), which are detected on a DNA sequencer.

With the current rage over forensic biology, caused primarily by the television show *Crime Scene Investigation* (*CSI*), we decided to revive D1S80 analysis for student field trips. This required entirely revamping the way in which we analyze student DNA. First, we developed a fast PCR protocol that uses a two-temperature profile to cut cycling time in half—to just 30 minutes. Second, we shifted electrophoresis to an Agilent microfluidics chip that separates D1S80 alleles in 2 minutes per sample. Amplified DNA fragments pass through microfluidic channels and each digital output displays the concentration and size of each allele. A set of 12 samples is entirely analyzed in 30 minutes, including generating a PDF genotype report for each student. The electropherogram result is identical to a single channel of the CODIS panel used in criminal investigations.

As a result, students can have a complete experience in forensic DNA analysis—from introduction, to buccal DNA isolation, to PCR amplification, to electrophoretic analysis, to analysis of electropherograms and population genetics—in a single 3.5-hour lab session. D1S80 forensic analysis will be routinely available in 2008 for classes visiting the Dolan DNALC and Harlem DNA Lab. We believe that we will be first group worldwide to make this sort of automated DNA analysis available to large numbers of precollege students.

This state-of-the-art technology was made possible through two important donations. Leo Brizuela, Director of Biology at Agilent Technologies and a former CSHL employee, arranged the donation of a Bioanalyzer valued at $16,280. Tom White, Chief Scientific Officer of Celera, arranged the donation of a super-fast Veriti thermal cycler, valued at $7,995, from Applied Biosystems. Dr. White was an early supporter of our work to popularize PCR, helping us to obtain an educational license and donating several of our first PCR machines in the early 1990s.

During the year, we also continued to support a second forensic DNA experiment by providing free sequencing of student DNA samples submitted by biology classes worldwide. This project analyzes the DNA found in the cell's energy-producing organelle, the mitochondrion (mt). With thousands of copies



CSI-DNALC: Using the new Bioanalyzers, gel images and electropherogram results are available to each student.

of the mt chromosome per cell, as opposed to two copies of nuclear chromosomes, mt DNA is abundant in tissue samples. Thus, mt DNA is the DNA of last resort in forensic cases where tissue remains are very old or degraded. Forensic analysis usually focuses on the noncoding "control" region of the mt chromosome that rapidly accumulates mutations and, thus, is highly variable between people. This is the same DNA analyzed by National Geographic's *Genographic Project* and described in the popular book *The Seven Daughters of Eve*. Notably, mt DNA was used to identify the remains of the Romanov royal family and to determine the relationship of Neandertal fossils to modern humans.

Using DNALC protocols or ready-to-use kits available from several science suppliers, students use PCR to amplify a 440-nucleotide sequence of their mt control region and send their amplified samples to the DNALC via overnight mail. The student samples are prepared for sequencing by college interns Alina Duvall (Hofstra University) and Jennifer Aiello (Long Island University at C.W. Post). The samples are then sent to the CSHL sequencing center in Woodbury, where they are processed on an Applied Biosystems 3730xi Genetic Analyzer. The finished sequences are uploaded to a student DNA database at the DNALC's *BioServers* Internet site (www.bioservers.org). The *Sequence Server* site provides tools to perform comparative analysis of mt DNA sequences from world populations, as well as ancient samples, including Neandertal and Otzi "the ice man."

In 2007, we sequenced 6303 student DNA samples submitted by high schools and colleges from 34 states and British Columbia. On average, results were posted on the *Sequence Server* site within 16 days of receipt. The free *Sequencing Service* is made possible by the donation of sequencing reagents by Applied Biosystems.

Visitation and Student Instruction

Annual visitation reached 41,897 in 2007. This included 19,973 students who conducted experiments at the DNALC or DNALC *West* and 10,629 who received in-school instruction by DNALC staff members. More than half of all visitors (20,909) were middle school students who conducted experiments under the

banner of *Genetics as a Model for Whole Learning* (*GMWL*). Cablevision's *Long Island Discovery* multimedia show drew 6750 visitors, primarily fourth graders studying Long Island history.

A record 971 students participated in week-long summer camps conducted at the DNALC, DNALC *West*, Central Islip High School, Sarah Lawrence College (Bronxville), and Aspen High School (Colorado). This great result was facilitated by a new system for online registration and payment via PayPal. More than 60% of parents took advantage of this streamlined way of enrolling their children, saving valuable staff time.

The roundworm *C. elegans* made its first appearance in summer camps this year. In *Fun with DNA*, 5th to 6th grade students used stereomicroscopes to compare wild-type and mutant worms, illustrating that gene mutations cause visible changes in traits. In *Genetic Horizons*, 8th to 9th graders used RNAi to induce phenotypes and bioinformatics to learn more about the genes they were disrupting. These were likely the first middle school students in the world to use RNAi!

Fifth grade students observe *C. elegans*.

We also initiated a collaboration with *Project Grad Long Island*, a nonprofit organization that works within economically disadvantaged communities to increase high school graduation rates. *Project Grad* provided scholarships and daily bussing for 26 middle school students from the Westbury school district to attend a week-long camp at DNALC *West*. This custom experience—combining hands-on labs from our popular *Fun with DNA* and *World of Enzymes* camps—was designed to build a strong foundation in basic genetics and molecular biology for future high school work.

The annual *Great Moments in DNA Science* honors seminar series drew 437 students for insights into modern biological research. Dr. Elizabeth Murchison explained how scientists are attempting to understand the genes involved in a mysterious transmissible cancer in Tasmanian devils, with the hope of saving this unique animal from extinction. Dr. Bill Keyes discussed an intriguing theory about how cellular mechanisms aimed to protect our bodies from cancer may also contribute to aging. In the final lecture, Dr. James Watson presented anecdotes from his latest book about how to succeed in science. *Saturday DNA!* continued to be popular with both students and adults, drawing 350 participants for hands-on sessions ranging from "Jellyfish Genes" to "CSI: Learning Center."

In its second year, our partnership with Cold Spring Harbor High School (CSHHS) provided a challenging experience for ten students. The year-long "capstone" course was coinstructed by DNALC staff and CSHHS biology teacher Scott Renart, with students coming on alternate days to the DNALC for their final two class periods. The course emphasized critical thinking and included experiments and independent projects across a range of biological systems. A bacterial unit focused on recombinant DNA technology and gene manipulation; a plant unit analyzed transgenes in genetically modified food and newly sequenced genes in rice; human-based experiments used molecular tools to examine human origins and genetic variation; and work with *C. elegans* introduced the cutting-edge RNAi technique.

During the winter, graduate students from the Watson School of Biological Sciences completed 12 half-day teaching sessions during their annual rotation at the DNALC. During the first phase of training, each pair of students observed a DNALC instructor at work and organized a lesson plan that integrated their own perspectives. In the second phase, each pair worked closely with a DNALC instructor to coteach several labs, developing their communication skills and preparing them for independence. During the final phase, each pair was responsible for presenting an entire lab, under the close observation of a DNALC staff member. After completing this cycle for middle and high school classes, students chose three additional lessons to independently demonstrate instructional and classroom management skills.

Faculty Training

In 2007, more than 500 educators participated in a variety of professional development activities conducted at sites around the United States and Europe. With sponsorship primarily from the NSF, we collaborated with 13 host institutions to conduct 1.5–5-day workshops on plant genomics, bioinformatics,

and RNAi. Demand for these workshops remained strong, with 384 applicants competing for 226 spaces. Five of these workshops were conducted at historically Black (HBCU) or Hispanic (HACU) institutions, where 36% of participants were Black or Hispanic. Overall, 27% of participants at offsite workshops were underrepresented minorities, mirroring their proportion among American residents (2005 Census Figures). An additional 250 educators attended workshops at professional meetings in the United States and Europe, which covered topics including neurobiology, bioinformatics, the molecular genetics of taste and smell, detecting GM foods by PCR, DNALC online tools for education, and forensic analysis of the Romanov family remains.

Twenty-five high school teachers, representing 14 states and Singapore, attended the annual *Amgen Leadership Symposium in Human and Genomic Biology*. Initiated in the mid-1990s with NSF support, the *Leadership Symposium* is the DNALC's capstone course, aimed at providing super-order training for progressive biology teachers who emphasize hands-on instruction in genetics or biotechnology. During their 3-week residence, participants lived and breathed science on the CSHL campus, where they walked in the footsteps of Nobel laureates.

The curriculum began with 2 weeks of the DNALC's latest experiments on RNAi, human DNA polymorphisms, and bioinformatics. Participants explored RNAi in the roundworm *C. elegans*, first observing mutant phenotypes and learning basic worm care, progressing to simple methods of inducing RNAi, and then to the construction of their own reagents to "knock down" a gene of their choice. Participants examined their own DNA for several polymorphisms. Point mutations in mitochondrial DNA and an *Alu* insertion on chromosome 16 provided an entry point into the study of human populations and early migrations of our species. A mutation in a taste receptor correlated with the ability to taste the bitter chemical phenylthiocarbamide (PTC), showing the relationship between genotype and phenotype. Participants used a variety of online tools and DNA sequence analysis software to find gene elements in DNA sequences, to find genes in databases, and to compare computer predictions with biological evidence. The *Symposium* concluded with a week of independent or group projects that included optimizing RNAi methods for classroom instruction, screening a variety of supermarket foods for DNA evidence of genetic modification, annotating newly discovered genes from the rice genome, and creating video introductions to RNAi experiments. The experience was further enhanced by seminars by CSHL plant biologist Robert Martienssen, CSHL structural biologist Leemor Joshua-Tor, and Nobel laureate James Watson.

Leadership participants strike a pose with James Watson and Learning Center staff on the DNALC terrace.

The DNALC continued to provide teacher training under its long-term collaboration with the Singapore Ministry of Education. Initiated in 2000 with a visit by Minister Rear Admiral Teo Chee Hean, the collaboration was guided by a memorandum of understanding through which licensed DNA learning centers were established at the Singapore Science Center and National Institute of Education. Two junior college teachers and an instructor from the Singapore Science Center participated in the *Leadership Symposium*, and five elementary teachers came in November for a 2-week attachment. The attachment included hands-on lab work, lab preparation, and instructional pedagogy. The Singaporean teachers observed and cotaught labs alongside DNALC instructors and visited several local elementary schools. Each teacher developed a plan for translating their experience into hands-on instruction for Singaporean students.

Internet Visitation and Development

Visits to DNALC Internet sites rose to 7.14 million in 2007. The brightest spots were the *Image Archive on the American Eugenics Movement*, which more than doubled to 731,913 visits, and *Inside Cancer*, which increased 63%, to 196,138 visits. We began tracking the amount of data downloaded from our sites in February and were impressed to see that our content-based sites averaged more than 47 gigabytes (GB) per month. According to answers.com, one GB of data is roughly equivalent to 1000 novels at 100,000 words each, 18 hours of MP3 music, or 12 hours of Flash video. *DNA Interactive*, our richest multimedia site, came out on top of this statistic, serving a total 1200 GB in the year.

Educators, students, and scientists present their idea of a gene on *Dynamic Gene*'s home page.

We launched two new Internet sites in 2007. *Silencing Genomes* offers laboratory and bioinformatics exercises to introduce students to RNAi and its effects on *C. elegans* anatomy and behavior. *Dynamic Gene* introduces modern concepts of gene analysis and provides "doable" student projects using research tools to analyze new genome data. *RedOrbit.com*, a science community site, recognized *Silencing Genomes* (June 13) as well as *Inside Cancer* (August 27) as "Red Hot Sites of the Day."

Internet site (content-based sites and laboratory/ bioinformatics sites)	Average visit (min)	Average monthly bandwidth	Visits in 2007	% Change from 2006
Gene Almanac	8:33	66.45 GB	1,923,185	–16.37
DNA from the Beginning	8:32	29.68 GB	1,451,572	–1.99
Your Genes, Your Health	7:40	46.97 GB	962,151	–13.72
DNA Interactive & myDNAi	8:24	100.43 GB	1,459,865	15.26
Image Archive on the American Eugenics Movement	16:28	11.83 GB	731,913	120.86
Inside Cancer	7:09	30.08 GB	196,138	62.98
BioServers	16:10	2.29 GB	251,597	10.13
Genetic Origins	7:04	1.59 GB	132,748	–12.06
Greenomes	3:27	0.40 GB	9,443	10.60
Dynamic Gene	6:20	0.60 GB	7,986	n.a.
Silencing Genomes	8:11	0.44 GB	12,168	n.a.
DNALC Kits/Carolina Collaboration	15:26	1.60 GB	4,396	n.a.
All sites			**7,143,172**	**2.00**

We were disappointed, however, by the decreased visitation to our home page, *Gene Almanac*, and to *Your Genes, Your Health* and *DNA from the Beginning*. The decrease at *Gene Almanac* most likely reflects the fact that increasing numbers of visitors now reach our family of Internet sites directly by Internet searches, without going by way of the home page. We believe that the decreases at *Your Genes, Your Health* and *DNA from the Beginning* relate to the fact that content created in Adobe's multimedia authoring tool *Flash* is less "visible" to search engines than html-based sites. As the volume of Web content increases exponentially and people increasingly rely on Internet searches, we will need to solve this problem of search engine visibility.

We have already undertaken several simple strategies: registering site maps on Google; creating html directories of the sites on our servers; and cross-featuring newer sites on other more established sites or within the *Resources* section of *Gene Almanac*. We are also working on more involved solutions to the visibility problem. Beginning in July, our sites benefited from "piggybacking" on a Google AdWords grant. AdWords is Google's advertising program that displays "sponsored" links on the right side or top of a search results page. Through the grant, we receive these advertisements free of charge. For each DNALC Web site, a set of keywords is stored in the AdWords account. Each time one of the keywords is typed into Google's search box, a short ad for the site is displayed and logged as an "impression." If the searcher clicks on our ad, it counts as a "click-through" and a cost per click of up to $1.00 is applied. *DNA from the Beginning* is our most successful site on AdWords, with 2,608,437 impressions and 43,356 click-throughs. Not surprisingly, 79.28% of these click-throughs resulted from a search of "DNA." All DNALC sites received 5,901,454 impressions and 62,740 click-throughs, which would have cost $37,106 for a commercial enterprise to purchase from Google. A quick analysis of search engine referrals in the first half of 2007 (prior to initiating the AdWords program) versus the second half showed an 2% increase.

In addition, the increasing use of sophisticated search engines to find specific information runs counter to narrative structure of our older content sites. Thus, we are decomposing all our Internet sites into content "atoms" that can be searched for and viewed independently. Using the content management system (CMS) created for *Genes to Cognition Online*, each item is equipped with meta-tags that make it more visible to search engines and more useful to end users. Each animation and video item is saved as an individual atom of content with a CMS entry including keywords, media type, and educational uses and context. We are also linking the atomized content to a specialized search tool/viewer that will soon be a major feature of a redesigned *Gene Almanac* portal. We look forward to the day when our CMS, meta-tag, and search tool/viewer will deliver exactly what any visitor is looking for.

Genes to Cognition Online

A cornerstone of DNALC's strategy to maintain its leadership in online science education has been to stay in touch with thought leaders in research and education. Thus, late in 2006, we hosted *New Horizons in Internet Site Development*, a 2.5-day conference that brought together experts in cognitive and neural science, learning theory, and technology to determine how research can support science teaching using advanced Internet technologies. In 2007, we responded to insights from the conference with a number of content and design initiatives to improve *Genes to Cognition* (*G2C*) *Online*, an Internet site on the molecular basis of human thinking and disorders of thinking that we are developing under grants from the Dana Foundation and the William and Flora Hewlett Foundation.

Insights were collated to produce a working paper of performance indicators, intended to guide project development. One of the main recommendations was the need to create a smooth interface for interacting with content. We responded by creating a networked map that builds on open-source code recently developed by The Media Lab at the Massachusetts Institute of Technology. The map displays site content as a network of dimensional content "atoms." These atoms cluster around major nodes such as schizophrenia, autism, or neuroimaging and are direct translations of expert concept maps we elicited from science experts in 2006. The atom map provides an enticing interactive entrée to *G2C Online* content, allowing users to visualize relationships between content items. Rolling over an atom provides a preview of the content item—such as "The schizophrenic brain," below—which can be accessed by a single click. Users can also make use of traditional online mapping tools such as zoom and pan.

Another step in the evolution of the *Genes to Cognition* Internet site; users will navigate the site using the network map. Project staff expect to launch the site in 2008.

We also addressed search and navigation functions. A familiar problem with Internet sites built in *Flash* is that users encounter difficulty switching between recently visited items. Thus, we developed a history function that keeps track of content that makes for easy forward/backward navigation.

Interactivity with content was a major theme in our Advisory Panel meeting, which we convened in October. We followed up on the Panel's recommendation to make the atom map the primary tool for accessing content. Now, when users visit *G2C Online*, their first interaction with material will be with the map, which provides a visual impact. Further interaction with the atoms should encourage users to make associations between different areas of research.

Following the meeting, we agreed to build two major interactive animations, which will be the centerpiece of the *G2C Online* project—an interactive brain map and a three-dimensional action potential animation. We are currently in negotiations with an animation studio, AXS-3D, to develop the content. The 14 advisors and staff members who attended the annual meeting were pleased with the free-flowing and frank discussions and willingness to take changes on board.

Project development was additionally guided by a series of evaluations conducted with the Center for Children and Technology. We used two different types of assessments, which enabled us to troubleshoot potentially problematic content and design issues. One-on-one qualitative evaluations with students from Philadelphia and New York City high schools allowed us to assess how students independently explore *G2C Online*. We learned that Internet site users generally cannot view animations and listen to narration simultaneously. In response, we restructured relevant items to make animation and narration features sequential as opposed to parallel. The second arm of the evaluation program consisted of a usability study conducted with 92 high school students from Long Island and Philadelphia. We identified problems with functionality and slow-loading media items that were addressed in a subsequent revision of the site.

G2C Online content continues to evolve. During the year, we edited more than 200 video items and made many available on our demo site. We collaborated with the Dana Foundation to repackage and host 68 articles from www.dana.org. In addition, we stepped up production of educational tools and created a number of innovative media, including:

∞ *Fly School*, an interactive game that allows students to structure experiments to test memory in normal and mutated fruit flies. This module was developed in collaboration with CSHL researcher Tim Tully.

∞ *Model Center*, a bioinformatics tool that allows students to compare protein sequences in different model organisms.

∞ *Chromosome Map Viewer* is also a bioinformatics tool that allows students to examine the chromosome locations and biological functions of genes associated with cognitive disorders.

Staff and Interns

In October, DNALC Assistant Director Uwe Hilgert left to become Assistant Dean of the Watson School of Biological Sciences. Reared on a riverboat in Germany, Uwe received a Ph.D. from the Max-Planck Institute for Plant Breeding in Cologne and conducted postdoctoral research at the University of Arizona. Uwe started at the DNALC in 2000 as a high school instructor and then quickly became leader of bioinformatics teacher training under an HHMI grant. As Assistant Director, beginning in 2005, Uwe assumed responsibility for all of the DNALC's professional development activities, in addition to overseeing the instructional staff and the operations of DNALC *West*. Uwe's stamina, dedication, and attention to detail will be sorely missed.

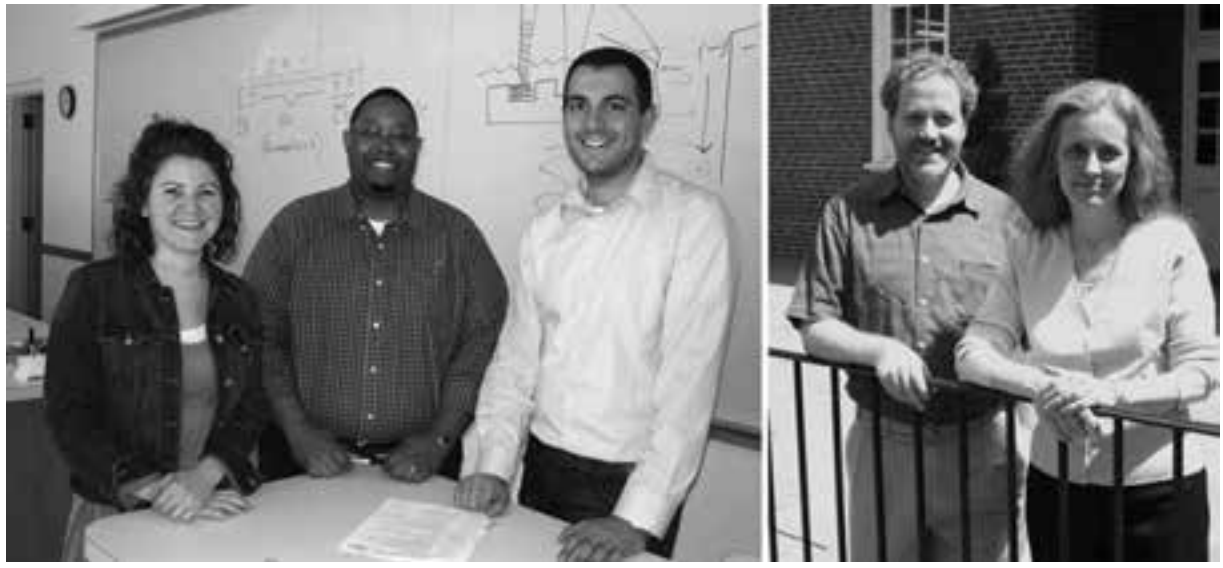
After Uwe's departure, the assistant directorship was split into two positions. Amanda McBrien became Assistant Director for Instruction, and Bruce Nash became Assistant Director for Science. Amanda joined the DNALC 1998, and under her direction, the middle school program has grown by 65% and become a major source of annual operating income. She brings to her new position intimate knowledge of local school systems and experience as an expert trainer. Amanda is responsible for the smooth running of student programs, including field trips, in-school instruction, summer camps, and Watson School teaching rotations. Bruce Nash earned his Ph.D. in molecular genetics at the University of Toronto and conducted postdoctoral research on cell division in *C. elegans* at the University of Oregon. He was specifically recruited to bring *C. elegans* and RNAi technology to the DNALC. Bruce is responsible for technical support of the lab and DNA sequencing programs, development of new labs, and external teacher training.

At mid year, we lost three innovative and inspirational instructors. Greg Chin returned to his native San Jose to spend time with his family. After receiving a Ph.D. in Developmental Biology from Stanford University and conducting postdoctoral research at the DNAX Research Institute, Greg was recruited in 2005 (along with Bruce Nash) to jump-start our RNAi effort. David Gundaker moved to Dover, Massachusetts, with his family to accept a full-time teaching position at the Charles River School. He came to the DNALC in 2005 with master's degree in education and real-world teaching skills developed in middle schools in Colorado and Long Island. Laura Johns took a position with the technical support staff of the biotech company, Invitrogen. With a bachelor's degree in genetics and graduate studies in marine chemistry, Laura brought the technical skills needed to administer our DNA sequencing service.

We were pleased to bolster the DNALC's instructional staff by welcoming two high school instructors, Jermel Watkins and Brian Lang, and one middle school instructor, Jennifer Cutillo. We were especially happy to welcome back Jermel, who began his science career as a DNALC intern from 1994 to 2000. During the summers, he also worked alongside his father and DNALC collaborator Jerry Watkins, teaching *DNA Science* to minority students from his home school, Central Islip. Jermel went on to earn his Ph.D. in molecular and cellular pharmacology at Stony Brook University. After receiving his bachelor's degree from South Hampton College in marine microbiology, Brian worked as a lab technician at Pall Corporation. He went on to receive a master's degree in secondary education and New York State certification. After receiving her bachelor's degree in biology and secondary education from Providence College, Jennifer taught life science and biology for 2 years in Boston.

The *BioMedia* Group received a boost when Stephen Blue started late in the year as a part-time multimedia designer. Stephen is a graduate of Parsons School of Design, where he is an adjunct computer instructor.

In the spring, the administrative staff lost two strong clerical workers. Nancy Daidola left to pursue personal goals, and Stacy Leotta took on a position in the CSHL Office of Sponsored Programs. Prior to joining the DNALC in 1999, Nancy built her skills in administration at Grumman Aerospace and as real estate agent with Coldwell Banker Sammis. Nancy performed a myriad of administrative tasks that kept the



New staff at the DNALC in 2007 (*left to right*): Jennifer Cutillo, Jermel Watkins, Brian Lang, Stephen Blue, and Valerie Meszaros

DNALC running smoothly. Stacy joined the DNALC in 2005, using her database skills to aid in the computerization of the front office. Valerie Meszaros arrived in the spring to fill the open administrative position left by Stacy. Valerie, a Long Island native, was formerly an editor and writer for Healing Tao Press.

Our internship program continues to offer Long Island high school and college students the opportunity to gain practical laboratory experience. Joining the intern program in 2007 were Charmaine Browne (Westbury High School), Victoria Grace (Oyster Bay High School), Emily Lopes (Commack High School), and Stephanie Parascandolo (Half Hollow Hills High School). Tara Dolan (University of Miami) and Danielle Sganga (Vanderbilt University), who were graduates of the first CSH Partnership Program, joined with current Cold Spring Harbor High School student Rachel Gellerman to work on RNAi and *C. elegans* under the guidance of Bruce. New members of the intern team joined returning high school students Seth Schortz (Half Hollow Hills High School) and Matthew Levy, Hal Mutlu, Arielle Scardino, Nick Wilken, and Janice Yong (all of Kings Park High School). Several interns returned from college to assist with summer workshops: Benjamin Blond (Amherst College), Joseph Hakoopian (Cornell University), Alexandra Sloane (Loyola College), and Margarita Varer (SUNY Binghamton).

High School intern Rachel Stephan (Kings Park), was a semifinalist in the 2007 Intel Science Talent Search for her project on eliminating polychlorinated biphenyls from the environment. Rachel won first place in the environmental science category of the International Science and Engineering Fair and presented her project in New Mexico.

In August, we bid farewell to the following interns as they left for their freshman year at college: Matt Giambrone (Walt Whitman High School) to Cornell University; Carissa Maurin (Lynbrook High School) to Monmouth University; Ronnie Morasse (Plainedge High School) to Stevens Institute of Technology, and Brittany Woods (Cold Spring Harbor High School) to Boston College.

Expert Advisors and Corporate Support

We are lucky to have high-level support from two advisory bodies: the DNALC Committee and the Corporate Advisory Board (CAB). The Education Committee formulates policy and assists with strategic planning, whereas the CAB provides a liaison to the Long Island business community. The CAB conducts an annual golf tournament and a fund campaign that are the major sources of unrestricted funds to support the DNALC.

DNALC Committee

Chairperson: **Laurie Landeau, V.M.D.**

Marion Wood Ahmed
Lori Bressler
Edward A. Chernoff
Lola N. Grace
Suzanne Kleinknecht
Suzanne Leeds
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John C. Kean III, Kean Development Corporation
Norman Kelker, Enzo Life Sciences
Robert Keller, Keyspan Foundation
Teresa Kemp-Zielenski, United Way of Long Island
Scott Livingston, Axiom Capital, Livingston Group
James E. Mattutat, Ingleside Investors, Inc.
John Passarelli, MD
Patricia Petersen, Daniel Gale Sothebys Real Estate
Frank Posillico, Rose Racanelli Realty/Aireco
Christiaan B. Reynolds
William D. Roche
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, AGI Dermatics
Hans Zobel, Festo Corporation

David A. Micklos
Executive Director

2007 Workshops, Meetings, and Collaborations

January 2–5	Site visit by Debra Burhans, Canisius College, Buffalo, New York
January 4	Site visit by Magda Hayden, Bob Isakkson, Elena Perrez, and Peter Quick, Bank of America, Melville, New York
January 14	Site visit to Gates Foundation, Washington, D.C.
January 16	Site visit by Stephanie Tzall and Charlie Pamaro, Brooklyn Technical High School, New York
January 18	Site visit by Mike Hyde and Rick Woychik, Jackson Laboratory, Bar Harbor, Maine
January 20	*Saturday DNA!*, "Designer Experiment" and "Macro Concepts of Microarrays,"
January 25	Site visit by Robert Hoppenstedt, Bethpage Federal Credit Union, Bethpage, New York
January 25	Site visit to Center for Children and Technology, New York, New York
February 1	Cold Spring Harbor High School Partnership Program, Independent Project Presentations, DNALC
February 5–8	Cancer Biomedical Informatics Grid (caBIG) Meeting, Washington, D.C.
February 8	Site visit by Christopher Hahn and Teresa Kemp-Zielenski, United Way Long Island
February 10	*Saturday DNA!*, "The Mystery of Anastasia Romanov" and "Mitosis, Meiosis, and Chromosomes, Oh Mei!," DNALC
February 13	Site visit by Exploratorium Center for Learning and Teaching, San Francisco, California
February 15	Site visit by Lewis Ranieri, Computer Associates and Hyperion, and Peter Quick, Bank of America, Melville, New York
February 23–24	NSF *Plant Molecular Genetics and Genomics* Follow-up Workshop, with Minority Fellow Mary Smith, Greensboro, North Carolina
February 28	Site visit by Syd Mandelbaum and WLIW Channel 21, Garden City, New York
March 1	Site visit by Walter J. Dillingham, Jr., Bank of America, New York, New York
March 5, 12, 19	*Reading Your DNA*, Cold Spring Harbor Central School District Adult Education Workshop, DNALC
March 7	*G2C Online* interview with Daniel Weinberger, National Institute of Mental Health, Bethesda, Maryland
March 16–17	NSF *Plant Molecular Genetics and Genomics* Follow-up Workshop, with Minority Fellow Muhammad Milan, Holly Springs, Mississippi
March 19–20	*G2C Online* interview with Seth Grant, Wellcome Trust Sanger Institute, Hinxton, United Kingdom
March 22	*G2C Online* interview with James Watson, CSHL
March 22	Site visit by Charterhouse School, Surrey, United Kingdom
March 23–24	NSF *Dynamic Gene* Workshop, Austin Community College, Texas
March 24	*Saturday DNA!*, "When Dinosaurs Roamed the Earth" and "CSI: Learning Center," DNALC
March 24	*DNA Extraction* Student Workshop, American Museum of Natural History, New York, New York
March 25–26	NSF *Plant Molecular Genetics and Genomics* Follow-up Workshop, with Minority Fellow Javier Gonzalez, Weslaco, Texas
March 26–April 1	National Science Teachers Association Annual Meeting, St. Louis, Missouri
March 27	Hewlett Open Education Meeting, Houston, Texas
March 27–28	NSF *Dynamic Gene* Workshop, St. Louis Science Center, Missouri
March 28	*G2C Research Programme* Meeting, London, United Kingdom
April 2	Site visit by Peter Jann, Life Science Zurich Learning Center, Zurich, Switzerland
April 3	Lymphatic Research Foundation meeting, Huntington, New York
April 6–7	NSF *Plant Molecular Genetics and Genomics* Follow-up Workshop, with Minority Fellow Olga Kopp, Orem, Utah
April 12	Search Engine Strategies Meeting, New York, New York
April 13	New York City Department of Education meeting, New York
April 13–14	NSF *Dynamic Gene* Workshop, Colorado Springs, Colorado
April 17	*Great Moments in DNA Science* Honors Seminar: "The Devils' Own Hell: Tasmanian Devil Transmissible Cancer," Elizabeth Murchison, CSHL
April 17	New York City Department of Education meeting, New York
April 19	*G2C Online* interview with William Kristan, University of California, San Diego
April 21	*Saturday DNA!*, "How Does Your Garden Grow?" and "Eureka! Art of Scientific Discovery," DNALC
April 21	*DNA Extraction* Student Workshop, American Museum of Natural History, New York, New York
April 23	*Great Moments in DNA Science* Honors Seminar: "Cancer and Aging: Getting the Balance Right," Bill Keyes, CSHL
April 25	Site visit from *The Times of Northport*, New York
April 25	Meeting with Tom Taratko, New York City Department of Education, New York
April 26, May 3, 10	*Reading Your DNA*, Adult Education Workshop, American Museum of Natural History, New York
April 30	*Great Moments in DNA Science* Honors Seminar: "Rules for Science," James Watson
May 1	Site visit by William Mak and Suet Ying Lee, Hong Kong Biotechnology Education Resources Centre Limited, China
May 7	New York State Department of Education Task Force meeting, New York Hall of Science, New York
May 9	Site visit by Doug Postl, Agilent Technologies, Wilmington, Delaware
May 11	New York City Department of Education meeting, New York
May 15	Site visit by Marion Conway, Roslyn Savings Foundation, and Tom Calabrese, Daniel Gale

	Sothebys Real Estate
May 17	The Human Genome Project: Library Training presentation "What DNA Says About Our Human Family," Baruch College, New York, New York
May 21	Cold Spring Harbor Laboratory Association meeting, DNALC
May 19	*Saturday DNA!*, "Fruit Fly Island" and "Tracking Ancient Treks," DNALC
May 24	Site visit by John Passarelli and Tom Gibbons, Notre Dame Club Development, New York, New York
May 31	Site visit by Robert Root, Brumsic Brandon, Oneil Eastmond, and Constance Clark, Westbury Public Schools; Robert Troiano, and Kim Arias, Project Grad; and Christopher Hahn and Terri Kemp Zielenski, United Way of Long Island
June 1–2	NSF *Dynamic Gene* Workshop, Canisius College, Buffalo, New York
June 2	*As the Worm Turns* Student Workshop, American Museum of Natural History, New York, New York
June 4–8	NSF *Plant Molecular Genetics and Genomics* Workshop, Oklahoma City, Oklahoma
June 5	Site visit by Bob Frehse and Ligia Cravo, Hearst Foundation, New York, New York
June 6	Site visit to Northport High School, New York, for *G2C Online*
June 6	Cold Spring Harbor High School Partnership Program graduation ceremony, CSHL
June 6, 13, 20	*Reading Your DNA*, Adult Education Workshop, American Museum of Natural History, New York, New York
June 7	Site visit by Kidgie Williams, Hospitality Committee for United Nations Delegations, Inc., and United Nations' family members, New York, New York
June 8	Site visit by Anne Marie Agnelli, Computer Associates International, Inc., Islandia, New York
June 9	*Saturday DNA!*, "Jellyfish Genes," DNALC
June 13–14	National Science Foundation *iPlant* Site Review, University of Arizona, Tucson
June 16	*The Iceman Cometh* Student Workshop, American Museum of Natural History, New York, New York
June 20	Site visit by Bob Keller, Keyspan Foundation, Hicksville, New York
June 21	*G2C Online* interview with Portia Iversen, Los Angeles, California
June 25–29	NSF *Plant Molecular Genetics and Genomics* Workshop, with Minority Fellow Gokhan Hacisalihoglu, Tallahassee, Florida
June 25–29	*DNA Science* Workshop, DNALC *Fun with DNA* Workshop, DNALC *West* *Fun with DNA* Workshop, DNALC *World of Enzymes* Workshop, DNALC
June 28	Site visit by Rod Miller, University of the Sciences in Philadelphia (USP), Russell DiGate, Philadelphia College of Pharmacy, and John Porter, Faculty Council, USP, Pennsylvania
July 2–8	*DNA Science Workshop*, Central Islip, New York *DNA Science* Workshop, DNALC *Fun with DNA* Workshop, DNALC *Green Genes* Workshop, DNALC *World of Enzymes* Workshop, DNALC *West*
July 9	Site visit by Anna Pascucci, University of Naples, and Liceo Scientifico Statale "G. Salvermini," Sorrento, Italy
July 9–13	*DNA Science* Workshop, DNALC *West* *Genetic Horizons* Workshop, DNALC *World of Enzymes* Workshop, DNALC
July 9–27	Amgen *Leadership Symposium*, DNALC
July 12	Site visit by Oscar Orrantia Vernaza, Pilar Morla de Orrantia, and Pilar Orrantia Quentin, Junta de Beneficencia in Guayaquil, Ecuador
July 12–13	National Science Foundation CCLI Grant Review, Arlington, Virginia
July 13	*G2C Online* interview with Thomas Nuhse, University of Manchester, United Kingdom
July 16	*G2C Online* interview with Jonathan Sebat, CSHL
July 16–20	*Fun with DNA* Workshop, DNALC *Green Genes* Workshop, DNALC *West* *Human Genomics* Workshop, DNALC
July 20	Site visit by Bill and Ursula Niarakis, Hoffman Center, Long Island, New York
July 23–27	*Green Genes* Workshop, DNALC *World of Enzymes* Workshop, DNALC
July 30–August 3	*DNA Science* Workshop, DNALC *Fun with DNA* Workshop, DNALC *West* *Fun with DNA* Workshop, DNALC *Genetic Horizons* Workshop, DNALC
July 31	Site visit by Bob Keller, Keyspan Foundation, Hicksville, New York
August 2	Site visit by Leslie Beller, Christine Knight, Edward Murphy, and Douglas Taylor, University of Virginia
August 6–10	*DNA Science* Workshop, DNALC *Green Genes* Workshop, DNALC *World of Enzymes* Workshop, DNALC *World of Enzymes* Workshop, DNALC *West*
August 9	Site visit by Fran Brennan, Veeco Instruments, Woodbury, New York
August 14	Site visit by Joe Novak, *G2C Online* Advisory Panel, and Joan Novak
August 14	Site visit by Jeff Carstens, Sovereign Bank, Long Island, New York

August 20–24	New York City Department of Education *DNA Science* and *PCR and Bioinformatics* Workshops, Brooklyn Technical High School, Brooklyn, New York
August 20–24	*DNA Science* Workshop, Sarah Lawrence College, Bronxville, New York
August 21	Site visit by Karen Agostisi and Tricia Russell, Office of Congressman Steve Israel
August 23–24	Hackathon Meeting, Chicago, Illinois
August 28	Bioinformatics Seminar, International Chromosome Conference, Amsterdam, The Netherlands
August 31	*PTC Bioinformatics*, International Chromosome Conference, Vienna Open Lab, Vienna, Austria
August 31	Site visit by Bob Keller, Keyspan Foundation, Hicksville, New York
September 24	Site visit by Dmitri Dumas, Diefendorf Capital Planning Associates, Locust Valley, New York
October 11	Site visit by Roy Crawford, The University of Waikato, New Zealand, and Renee Crawford
October 13	President's Council Meeting at DNALC, Sydney Brenner, Salk Institute; Phil Sharp, McGovern Institute for Brain Research at Massachusetts Institute of Technology; Gregory Hannon and Marja Timmermans, CSHL
October 15	*G2C Online* Advisory Panel Meeting, DNALC
October 20	*Saturday DNA!*, "Breaking Up is Hard to Do" and "The Ins and Outs of Cancer," DNALC
October 22–24	Howard Hughes Medical Institute Precollege Program Director's meeting, Washington, D.C.
October 31	Enhancing Collaborative Leadership for Improved Performance in Science Education, (ECLIPSE), Albany, New York
Oct. 31–Nov. 2	Novartis Exploratory Oncology Development Group Educational meeting, DNALC
November 5	Site visit by Ed Lee, *Apollo* Developer, Berkeley Bioinformatics and Ontologies Project, Lawrence Berkeley National Labs, California
November 7	Site visit by Yoshi Ishikawa, Masahiro Nei, and Lee Shuett, Nikon Instruments
November 12	Site visit by Bob Keller, Keyspan Foundation, Hicksville, New York
November 16–18	Howard Hughes Medical Institute Genomics Education Meeting, Chevy Chase, Maryland
November 17	*Saturday DNA!*, "Yeasty Beasties" and "Beyond DNA," DNALC
November 27–28	NSF *Dynamic Gene* Workshop, National Science Foundation, Decatur, Georgia
November 28	National Association of Biology Teachers, "Sense in Molecules," Atlanta, Georgia
November 29	National Association of Biology Teachers meeting, "Detecting GM Food by PCR," "Dynamite WWW Teaching Resources," Atlanta, Georgia
December 5	Presentation to New York State Genetics Task Force, New York University Medical Center
December 7	NSF *Dynamic Gene* Workshop, Caltech, Pasadena, California
December 7	Singapore Primary Teachers Attachment, DNALC
December 15	*Saturday DNA!*, "Iceman" and "The Buzz on Bees," DNALC
December 21	*G2C Online* interview with Jon Lieberman, Psychologist, Hampton Bays, New York

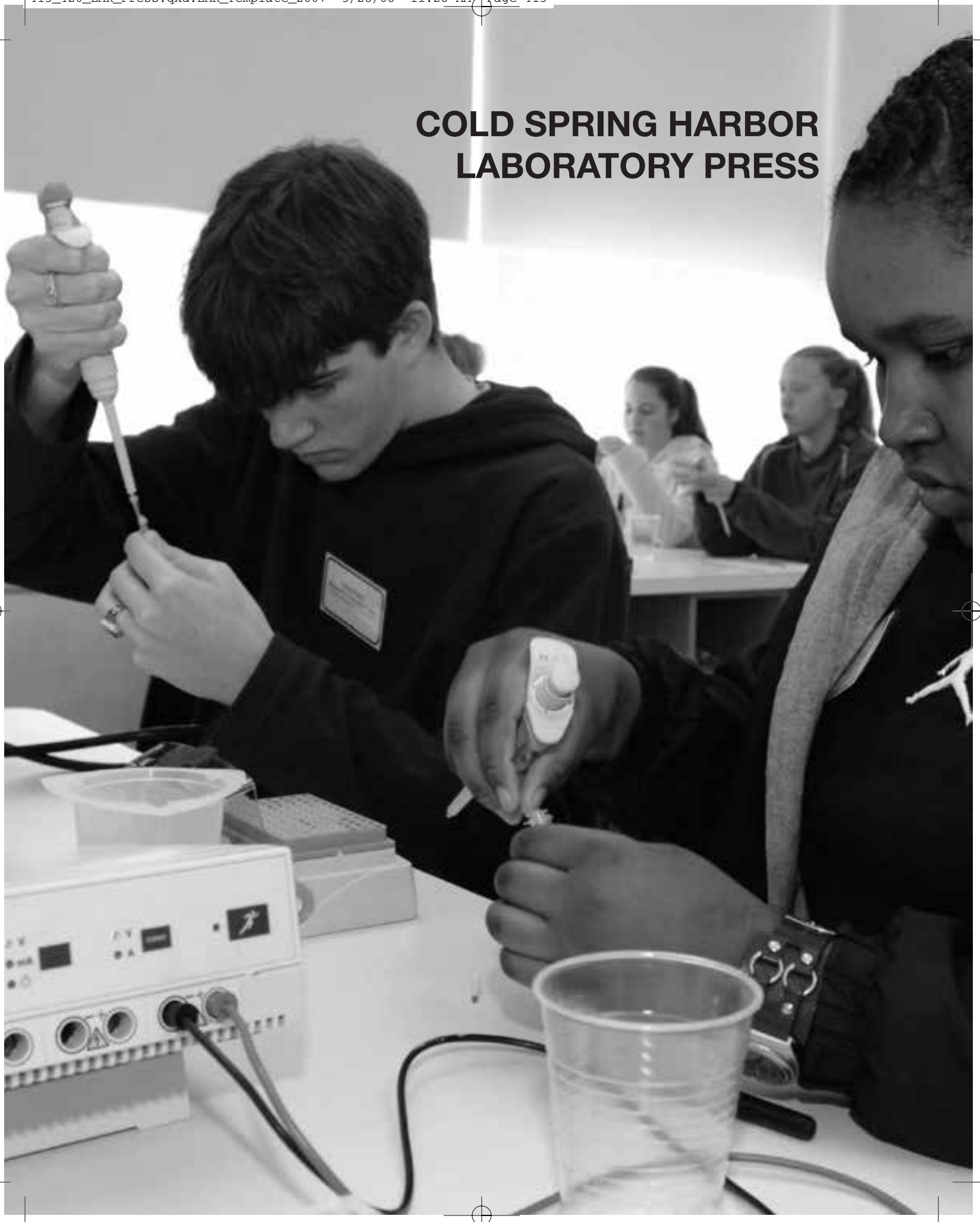
Sites of Major Faculty Workshops 1985–2007

Key: *Middle School* High School **College**

ALABAMA	University of Alabama, Tuscaloosa	1987–1990
ALASKA	University of Alaska, Fairbanks	1996
ARIZONA	Tuba City High School	1988
ARKANSAS	Henderson State University, Arkadelphia	1992
CALIFORNIA	**California State University, Fullerton**	**2000**
	California Institute of Technology, Pasadena	**2007**
	Canada College, Redwood City	1997
	City College of San Francisco	2006
	Contra Costa County Office of Education, Pleasant Hill	2002
	Foothill College, Los Altos Hills	**1997**
	Harbor–UCLA Research & Education Institute, Torrance	2003
	Los Angeles Biomedical Research Institute, Torrance	**2006**
	Laney College, Oakland	1999
	Lutheran University, Thousand Oaks	1999
	Pierce College, Los Angeles	**1998**
	Salk Institute for Biological Studies, La Jolla	2001
	San Francisco State University	**1991**
	San Jose State University	**2005**
	University of California, Davis	1986
	University of California, Northridge	**1993**
COLORADO	Aspen Science Center	2006
	Colorado College, Colorado Springs	1994, 2007
	United States Air Force Academy, Colorado Springs	**1995**
	University of Colorado, Denver	1998
CONNECTICUT	Choate Rosemary Hall, Wallingford	1987
FLORIDA	Armwood Senior High School, Tampa	1991
	Florida Agricultural & Mechanical University, Tallahassee	**2007**
	North Miami Beach Senior High School	1991

	University of Miami School of Medicine	2000
	University of Western Florida, Pensacola	1991
GEORGIA	Fernbank Science Center, Atlanta	1989, 2007
	Morehouse College, Atlanta	**1991, 1996–1997**
HAWAII	Kamehameha Secondary School, Honolulu	1990
IDAHO	University of Idaho, Moscow	1994
ILLINOIS	Argonne National Laboratory	1986, 1987
	University of Chicago	**1992, 1997**
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	Jefferson Parish Public Schools, Harvey	1990
	John McDonogh High School, New Orleans	1993
MAINE	Bates College, Lewiston	1995
	Foundation for Blood Research, Scarborough	2002
MARYLAND	Annapolis Senior High School	1989
	Frederick Cancer Research Center, Frederick	1995
	McDonogh School, Baltimore	1988
	Montgomery County Public Schools	1990–1992
	National Center for Biotechnology Information, Bethesda	2002
	St. John's College, Annapolis	*1991*
	University of Maryland, School of Medicine, Baltimore	**1999**
MASSACHUSETTS	Beverly High School	1986
	Biogen, Cambridge	2002
	Boston University	**1994, 1996**
	CityLab, Boston University School of Medicine	1997
	Dover-Sherborn High School, Dover	1989
	Randolph High School	1988
	Winsor School, Boston	1987
	Whitehead Institute for Biomedical Research, Cambridge	2002
MICHIGAN	Athens High School, Troy	1989
MINNESOTA	University of Minnesota, St. Paul	2005
MISSISSIPPI	Mississippi School for Math & Science, Columbus	1990–1991
	Rust College, Holly Springs	2006–2007
MISSOURI	Stowers Institute for Medical Research, Kansas City	2002
	Washington University, St. Louis	**1989, 1997**
NEVADA	University of Nevada, Reno	1992
NEW HAMPSHIRE	**New Hampshire Community Technical College, Portsmouth**	**1999**
	St. Paul's School, Concord	1986, 1987
NEW JERSEY	Coriell Institute for Medical Research, Camden	2003
NEW YORK	Albany High School	1987
	American Museum of Natural History	2007
	Bronx High School of Science	1987
	Canisius College, Buffalo	2007
	Columbia University	**1993**
	Cold Spring Harbor High School	1985, 1987
	Cornell University, Ithaca	**2005**
	DeWitt Middle School, Ithaca	*1991, 1993*
	DNA Learning Center	1988–1995, 2001–2004, 2006–2007
	DNA Learning Center	**1990, 1992, 1995, 2000**
	DNA Learning Center	*1990–1992*
	DNA Learning Center *West*	2005
	Fostertown School, Newburgh	*1991*
	Huntington High School	1986
	Irvington High School	1986
	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*

	The Rockefeller University	2003
	State University of New York, Purchase	1989
	State University of New York, Stony Brook	1987–1990
	Stuyvesant High School, New York	1998–1999
	Titusville Middle School, Poughkeepsie	*1991, 1993*
	Trudeau Institute, Lake Saranac	2001
	Union College, Schenectady	2004
	U.S. 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
	North Carolina School of Science, Durham	1987
OHIO	Case Western Reserve University, Cleveland	1990
	Cleveland Clinic	1987
	North Westerville High School	1990
OKLAHOMA	**Oklahoma City Community College**	**2000**
	Oklahoma City Community College	2006–2007
	Oklahoma Medical Research Foundation, Oklahoma City	2001
	Oklahoma School of Science and Math, Oklahoma City	1994
OREGON	Kaiser Permanente-Center for Health Research, Portland	2003
PENNSYLVANIA	Duquesne University, Pittsburgh	1988
	Germantown Academy	1988
SOUTH CAROLINA	**Clemson University, Clemson**	**2004**
	Medical University of South Carolina, Charleston	1988
	University of South Carolina, Columbia	**1988**
TEXAS	Austin Community College-Rio Grande Campus	2000
	Austin Community College-Eastview Campus	2007
	J.J. Pearce High School, Richardson	1990
	Langham Creek High School, Houston	1991
	Southwest Foundation for Biomedical Research, San Antonio	2002
	Taft High School, San Antonio	1991
	Texas A&M, AG Research and Extension Center, Weslaco	2007
	Trinity University, San Antonio	**1994**
	University of Texas, Austin	**1999, 2004**
UTAH	University of Utah, Salt Lake City	1993
	University of Utah, Salt Lake City	**1998, 2000**
	Utah Valley State College, Orem	**2007**
VERMONT	University of Vermont, Burlington	1989
VIRGINIA	Eastern Mennonite University, Harrisonburg	1996
	Jefferson School of Science, Alexandria	1987
	Mathematics and Science Center, Richmond	1990
	Mills Godwin Specialty Center, Richmond	1998
	Virginia Polytechnic Institute and State University, Blacksburg	**2005**
WASHINGTON	Fred Hutchinson Cancer Research Center, Seattle	1999, 2001
	University of Washington, Seattle	**1993, 1998**
WASHINGTON, D.C	**Howard University**	**1992, 1996**
WEST VIRGINIA	Bethany College	1989
WISCONSIN	Blood Center of Southeastern Wisconsin, Milwaukee	2003
	Madison Area Technical College	**1999**
	Marquette University, Milwaukee	1986, 1987
	University of Wisconsin, Madison	1988, 1989
	University of Wisconsin, Madison	**2004**
WYOMING	University of Wyoming, Laramie	1991
AUSTRALIA	Walter and Eliza Hall Institute and University of Melbourne	1996
AUSTRIA	**Vienna Open Lab**	**2007**
CANADA	Red River Community College, Winnipeg, Manitoba	1989
ITALY	Porto Conte Research and Training Laboratories, Alghero	1993
	International Institute of Genetics and Biophysics, Naples	1996
PANAMA	**University of Panama, Panama City**	**1994**
PUERTO RICO	University of Puerto Rico, Mayaguez	1992
	University of Puerto Rico, Mayaguez	**1992**
	University of Puerto Rico, Rio Piedras	**1993**
	University of Puerto Rico, Rio Piedras	1994
RUSSIA	Shemyakin Institute of Bioorganic Chemistry, Moscow	1991
SINGAPORE	National Institute of Education	2001–2005
SWEDEN	Kristineberg Marine Research Station, Fiskebackskil	1995
	Uppsala University, Uppsala	2000
THE NETHERLANDS	**International Chromosome Conference, Amsterdam**	**2007**



COLD SPRING HARBOR LABORATORY PRESS

2007 PUBLICATIONS

SERIALS

Genes & Development, Vol. 21, 1–3406 (www.genesdev.org)
Genome Research, Vol. 16, 1–1952 (www.genome.org)
Learning & Memory, Vol. 14, 1–868 (www.learnmem.org)
Protein Science, Vol. 16, 1–2784 (www.proteinscience.org)
RNA, Vol. 13, 1–2388 (www.rnajournal.org)
Cold Spring Harbor Symposia on Quantitative Biology, Vol. 71: *Regulatory RNAs,* Bruce Stillman and David Stewart (eds.) (Available in print and online)
Cold Spring Harbor Protocols (www.cshprotocols.org)

LABORATORY MANUALS

Genetic Variation: A Laboratory Manual, Michael P. Weiner, Stacey B. Gabriel, and J. Claiborne Stephens
Single-Molecule Techniques: A Laboratory Manual, Paul R. Selvin and Taekjip Ha

HANDBOOKS

C. elegans *Atlas,* David H. Hall and Zeynep F. Altun
Safety Sense: A Laboratory Guide, Second Edition

MONOGRAPHS

Adult Neurogenesis, Fred H. Gage, Gerd Kempermann, and Hongjun Song
Invertebrate Neurobiology, Geoffrey North and Ralph J. Greenspan
Molecular Biology of Aging, Leonard P. Guarente, Linda Partridge, and Douglas C. Wallace
The TGF-β Family, edited by Rik Derynck and Kohei Miyazono

TEXTBOOKS

An Introduction to Nervous Systems, Ralph J. Greenspan
Evolution, Nicholas H. Barton, Derek E.G. Briggs, Jonathan A. Eisen, David B. Goldstein, and Nipam H. Patel
Molecular Biology of the Gene, Sixth Edition, James D. Watson, Tania A. Baker, Stephen P. Bell, Alexander Gann, Michael Levine, and Richard Losick

SCIENCE AND SOCIETY

Career Opportunities in Biotechnology, Toby Freedman
Max Perutz and the Secret of Life, Georgina Ferry
Phage and the Origins of Molecular Biology, The Centennial Edition, John Cairns, Gunther S. Stent, and James D. Watson

OTHER

CSHL Annual Report 2006: Yearbook Edition
CSHL Annual Report 2006
Banbury Center Annual Report 2006
Watson School of Biological Sciences Annual Report 2006

WEB SITES

http://www.genesandsignals.org: Web site to accompany *Epigenetics* with figures made available on our resource Web site
http://www.cshl-symposium.org; *Symposia*, Vol. 71 added to online *Symposia* Web site

A selection of recently published books

The journal publishing program

COLD SPRING HARBOR LABORATORY PRESS EXECUTIVE DIRECTOR'S REPORT

Cold Spring Harbor Laboratory's first-class biomedical research is complemented by an unmatched program of professional and public education in gene science. The vehicle for much of this service is the work of Cold Spring Harbor Laboratory Press. Its evolution into a highly respected, independent, professional publisher has produced six research journals, two of them among the most highly ranked in science; the world's most widely used laboratory manuals, including the most highly cited book in biology; a prestigious monograph series; the most historically important conference proceedings in molecular biology; and best-selling primers on professional skills development for scientists. These activities enhance the work of other divisions, such as the Dolan DNA Learning Center, and produce revenue that supports the work of the Laboratory as a whole and identifies its name with quality, reliability, and excellence.

In 2007, despite challenging market conditions, CSHL Press generated a substantial operating excess, with a preliminary 2007 margin of just under $500,000 on revenues of $10.371 million. The Press' total financial contribution to the Laboratory was more than $1.1 million.

Journals

Revenues from journal publishing continued to be a major contributor to this success, increasing by 4.5% from 2006. For the established titles, there was growth in both subscription base and advertising revenue. Our newest journal, *Cold Spring Harbor Protocols*, exceeded subscription revenue projections in its first full year and requests for institutional trial subscriptions continue to increase steadily, auguring well for this online-only publication's future. Although growth continues in most established markets such as the United States and Western Europe through targeted sales efforts to individual institutions, opportunities for expansion are also being sought in the world's emerging scientific communities, through new multijournal package subscription sales to institutional consortia in India, Korea, and countries in South America. The Press has been active in China for several years, and in 2007, a 3-year contract extension was signed with the National Science and Technology Library to continue Press journal access at more than 400 institutions in this country.

R. Sever, Acquisitions Editor, and D. Crotty, Executive Editor, *Cold Spring Harbor Protocols*

Throughout our program, the use of print copies is declining as individual scientists make more extensive use of Web-based editions and as institutional libraries focus on developing electronic collections. In 2007 alone, online use of articles in CSHL Press journals grew by 30%. In response, the online archives of *Genes & Development* and *Genome Research* were extended to include all issues ever published for each journal, and the decision was made to reduce the frequency of print issues of *Learning & Memory* to twice yearly in 2008, in anticipation of the journal's becoming an online-only publication in 2009. Print issues remain an important vehicle for advertisers, but opportunities for online advertising have increased, for example, with the distribution of tables of content by e-mail, and companies with products to offer our readers are developing an appetite for their use.

The utility of the editorial content of our journals continues to be enhanced by the continuous development of tools and features that result from our 10-year-long online-publishing partnership with Stanford University's HighWire Press. A new contract with HighWire was negotiated to enhance our online journal platform while reducing costs. New versions of our online journals will launch in Fall 2008 with innovative "Web 2.0" features, greater flexibility for content display, and customized functions that will assist both readers and authors.

Although its technology base is now vital to a journal's success, the quality of its content remains the most important differentiator. By all available metrics, *Genes & Development* and *Genome Research* are consistently ranked among the most distinguished journals in the fields of genetics and developmental and cell biology. *Learning & Memory*, with its more specialized emphasis on cellular and molecular approaches, is receiving more submissions than ever. And the journals we publish for scientific societies, *Protein Science* and *RNA*, also have encouraging usage trends.

I. Sialiano, M. Cozza—Press Development staff

Research published in our journals was featured widely in the popular press. Television, newspaper, and magazine coverage in 2007 included features on CNN, MSNBC, BBC, ABC News, and FOX News networks, *Time*, *Newsweek, US News & World Report, Forbes, Wired, Scientific American, National Geographic, New Scientist, San Francisco Chronicle, Washington Post, USA Today, International Herald Tribune, Boston Globe*, and *The Daily Telegraph*, as well as innumerable online news sources.

Books

Fourteen new books were published this year. As the list below indicates, they included timely and highly regarded manuals, handbooks, monographs, and history/biographies. The most keenly anticipated was the first book that the Press has independently developed for undergraduate teaching, *Evolution*, by Nicholas Barton, Derek Briggs, Jonathan Eisen, David Goldstein, and Nipam Patel. An innovative synthesis of evolutionary and molecular biology, *Evolution* was welcomed by reviewers and teachers as "superb," "outstanding," and "compelling." The book has a companion Web site (www.evolution-textbook.org) that contains extensive chapter notes, figures and tables from the book, and two online-only chapters, and is already in use at Harvard, Stanford, University of Pennsylvania, Oxford, and numerous other major universities in the U.S. and Europe.

Thirteen contracts were signed for new books that included advanced textbooks, manuals, monographs, and a number of titles promoting the public's engagement with science. Five agreements were signed with foreign publishers for translations of titles into Chinese, Japanese, and Korean, and two contracts were signed for foreign reprint rights.

Marketing and Sales

Just as the distribution and purchase of scientific information has changed dramatically throughout the past decade, our strategies, techniques, and in-house skills have followed suit. This year, the reorganization of our book and journal marketing and sales departments continued and the boundaries between their activities

B. Keen (*standing*), Finance Director, and K. Paterakis, Financial Assistant

melted still more. The work of these departments maintains the visibility of the Press, the Laboratory, and their educational products and services in an increasingly dense information world. A monthly electronic newsletter, now reaching 45,000 subscribers, announces new titles, notable journal articles, and Press news, and promotes other divisions of the Laboratory, including the Dolan DNALC, the Meetings and Courses program, and developments at the Library and Archives.

Investment in new staff and skills permitted significant enhancement of the CSHL Press Web site in 2007, with improved searching, Web indexing, a new layout, and new features such as RSS feeds. Beneath the surface, further technical improvements included tools for tracking usage and strengthening sales efforts, as well as dynamic intrapage updating for more efficient site maintenance. Podcasts and slide shows were developed for specific titles, including one that highlighted the life of Max Perutz, narrated by the author of *Max Perutz and the Secret of Life*, Georgina Ferry. And the Executive Editor of *CSH Protocols*, David Crotty, began a blog called *Benchmarks* that has a growing audience. Online features such as these offer readers, potential book buyers, and prospective authors the opportunity to learn more about the background and content of our publications. These additional efforts are associated with an increase in direct sales: The proportion of books sold through our Web site and our major online bookselling partner Amazon.com rose to a record high in 2007.

The Press published its first online information in 1997. Ten years later, what is most surprising is not that science publishing has changed, but that, fundamentally, so much has remained the same for so long. For much of the past decade, the Press, like most of its peers in academic publishing, has found ways to place on the Web information that was developed for print publication. At last, that way of thinking is giving ground to an online-first approach. A major goal for the Press is the invention of a means of publishing books online that places book content firmly in the information universe of individual scientists, with a viable business model. A strategy for achieving that goal has been developed and will be implemented in 2008, with the assistance of a strengthened editorial staff.

Meanwhile, journal publishing continues to struggle toward business models that combine financial viability with maximum possible access to content. Journals such as ours provide a vital community service that requires a full-time staff, including scientifically trained professionals. These journals lack the circulation to become supported through advertising alone and cannot rely solely on publication fees provided by elite scientists with support from wealthy foundations. Our response has been to offer authors in all journals that we publish the option to have their papers made freely accessible immediately on publication, in return for a fee. However, for the past 5 years, all papers published in *Genes & Development* and *Genome Research* have been made freely available only 6 months after publication and deposited in the PubMedCentral database of the

National Library of Medicine. In this respect, the Press is attempting to render its journals compliant with the public access requirements of organizations such as The Wellcome Trust, the Howard Hughes Medical Institute, and most recently the National Institutes of Health, while remaining financially viable and continuing to be valuable contributors to the Laboratory's economy.

Staff

In 2007, we welcomed new colleagues Delia King, Kathy LaForgia, Christina Lo, Robert Majovski, Stephanie Novara, Keri Paterakis, Ted Roeder, Patricia Serpe, and Lauren Schmidt. We said farewell, thanks, and good luck to Cher Mattes and Roberta Salant.

A complete list of staff members of the Press as of December 2007 is printed elsewhere in this volume. The Laboratory is privileged to have the contributions of such capable and hardworking professionals, with whom it is my pleasure to work. I wish to extend special thanks to the individuals who have the added responsibility of providing leadership in the diverse departments and projects of the Press: Jan Argentine, David Crotty, Alex Gann, Terri Grodzicker, Bill Keen, Wayne Manos, Marcie Siconolfi, Hillary Sussman, Linda Sussman, and Denise Weiss.

John R. Inglis
Executive Director

FINANCE

FINANCIAL STATEMENTS

CONSOLIDATED BALANCE SHEET
December 31, 2007
(with comparative financial information as of December 31, 2006)

		2007	2006
Assets:			
Cash and cash equivalents	$	25,576,470	23,563,414
Accounts receivable		2,669,325	2,995,007
Grants receivable		9,037,130	7,859,380
Contributions receivable, net		67,093,000	43,226,518
Publications inventory		4,033,100	3,720,386
Prepaid expenses and other assets		4,049,972	2,015,019
Investments		333,635,052	308,108,275
Investment in employee residences		5,446,047	5,557,353
Restricted use assets		3,991,338	3,598,745
Deposits with bond trustee		29,483,468	46,014,949
Land, buildings and equipment, net		159,204,452	130,942,408
Total assets	$	644,219,354	579,417,878
Liabilities and Net Assets:			
Liabilities:			
Accounts payable and accrued expenses	$	19,998,678	12,450,777
Deferred revenue		4,003,091	3,585,913
Bonds payable		97,200,000	97,200,000
Total liabilities		121,201,769	113,236,690
Commitment and contingencies			
Net assets:			
Unrestricted		219,853,852	213,562,751
Temporarily restricted		119,623,060	82,791,081
Permanently restricted		183,540,673	169,827,356
Total net assets		523,017,585	466,181,188
Total liabilities and net assets	$	644,219,354	579,417,878

CONSOLIDATED STATEMENT OF ACTIVITIES
Year ended December 31, 2007
(with summarized financial information for the year ended December 31, 2006)

	Unrestricted	*Temporarily Restricted*	*Permanently Restricted*	*2007 Total*	*2006 Total*
Revenue and other support:					
Public support—contributions and non-Federal grant awards	$ 18,471,699	46,374,702	6,786,787	71,633,188	59,431,937
Federal grant awards	30,887,232	–	–	30,887,232	33,128,165
Indirect cost allowances	19,634,082	–	–	19,634,082	20,258,404
Investment return utilized	18,299,207	–	–	18,299,207	10,741,551
Program fees	4,103,754	–	–	4,103,754	4,192,163
Publications sales	10,404,661	–	–	10,404,661	9,319,286
Dining services	3,640,595	–	–	3,640,595	3,780,431
Rooms and apartments	2,804,675	–	–	2,804,675	3,043,489
Royalty and licensing fees	1,092,311	–	–	1,092,311	693,345
Miscellaneous	910,490	–	–	910,490	931,666
Net assets released from restrictions	9,542,723	(9,542,723)	–	–	–
Total revenue and other support	119,791,429	36,831,979	6,786,787	163,410,195	145,520,437
Expenses:					
Research	65,315,923	–	–	65,315,923	62,822,726
Educational programs	12,017,459	–	–	12,017,459	13,565,968
Publications	10,241,091	–	–	10,241,091	9,351,363
Banbury Center conferences	1,342,587	–	–	1,342,587	1,124,415
Dolan DNA Learning Center programs	1,413,157	–	–	1,413,157	1,325,479
Watson School of Biological Sciences programs	3,206,295	–	–	3,206,295	2,973,896
General and administrative	13,111,199	–	–	13,111,199	13,619,460
Dining services	5,109,292	–	–	5,109,292	4,981,661
Total expenses	111,757,003	–	–	111,757,003	109,764,968
Excess of revenue and other support over expenses	8,034,426	36,831,979	6,786,787	51,653,192	35,755,469
Other changes in net assets:					
Investment return in excess of amount utilized	1,333,223	–	6,926,530	8,259,753	20,946,485
Change in fair value of interest rate swap	(3,076,548)	–	–	(3,076,548)	(3,370,108)
Increase in net assets	6,291,101	36,831,979	13,713,317	56,836,397	53,331,846
Net assets at beginning of year	213,562,751	82,791,081	169,827,356	466,181,188	412,849,342
Net assets at end of year	$ 219,853,852	119,623,060	183,540,673	523,017,585	466,181,188

CONSOLIDATED STATEMENT OF CASH FLOWS
Year ended December 31, 2007
(with comparative financial information for the year ended December 31, 2006)

	2007	*2006*
Cash flows from operating activities:		
Increase in net assets	$ 56,836,397	53,331,846
Adjustments to reconcile increase in net assets to net cash provided by operating activities:		
Change in fair value of interest rate swap	3,076,548	3,370,108
Depreciation and amortization	6,397,045	6,032,302
Net appreciation in fair value of investments	(17,332,229)	(24,403,523)
Contributions restricted for long-term investment	(9,760,257)	(30,918,132)
Changes in assets and liabilities:		
Decrease in accounts receivable	325,682	2,740,444
(Increase) decrease in grants receivable	(1,177,750)	492,837
Increase in contributions receivable	(26,406,799)	(31,856)
Increase in publications inventory	(201,657)	(851,626)
(Increase) decrease in prepaid expenses and other assets	(329,586)	535,210
Increase in restricted use assets	(392,593)	(372,207)
Decrease in accounts payable and accrued expenses	(560,202)	(1,042,172)
Increase (decrease) in deferred revenue	417,178	(651,641)
Net cash provided by operating activities	10,891,777	8,231,590
Cash flows from investing activities:		
Capital expenditures	(34,659,089)	(21,123,188)
Proceeds from sales and maturities of investments	84,438,846	97,347,685
Purchases of investments	(92,610,279)	(124,641,857)
Net change in investment in employee residences	88,191	434,677
Net cash used in investing activities	(42,742,331)	(47,982,683)
Cash flows from financing activities:		
Permanently restricted contributions	6,786,787	14,917,606
Contributions restricted for investment in land, buildings and equipment	2,973,470	16,000,526
Decrease in contributions receivable	2,540,317	4,383,403
Decreasse (increase) in deposits with bond trustee	16,531,481	(46,014,949)
Debt issue costs paid	–	(2,240,577)
Increase (decrease) in accounts payable relating to capital expenditures	5,069,642	(276,659)
Repayment of notes payable	(38,087)	(35,551)
Redemption of 1993 Suffolk IDA Bond	–	(3,000,000)
Issuance of bonds payable	–	55,000,000
Net cash provided by financing activities	33,863,610	38,733,799
Net increase (decrease) in cash and cash equivalents	2,013,056	(1,017,294)
Cash and cash equivalents at beginning of year	23,563,414	24,580,708
Cash and cash equivalents at end of year	$ 25,576,470	23,563,414
Supplemental disclosures:		
Interest paid	$ 3,812,914	2,614,630

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 private foundations, corporations, and individuals. The following section summarizes funding that occurred during 2007.

GRANTS January 1–December 31, 2007

COLD SPRING HARBOR LABORATORY

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2007 Funding*	
FEDERAL GRANTS					
NATIONAL INSTITUTES OF HEALTH					
Equipment Support	Dr. Lowe	03/01/07	02/29/08	$425,826	*
	Dr. McCombie	04/15/07	04/14/08	473,927	*
Program Project Support	Drs. Hannon/Krainer/Lazebnik/Lowe/ S. Muthuswamy/Myers/D. Spector/ Stenlund/Stillman/Tansey	01/01/07	12/31/11	4,358,993	*
	Dr. Stillman–CSHL Cancer Center Core	08/15/05	07/31/10	4,086,019	
	Drs. Lowe/Hannon/S. Muthuswamy	04/01/04	03/31/09	945,214	
	Dr. Huang	09/08/06	08/31/11	716,542	
Pioneer Award Support	Dr. Cline	09/30/05	07/31/10	827,584	
Merit Award Support	Dr. Malinow	05/01/92	04/30/10	656,267	
	Dr. Tonks	08/01/91	03/31/09	665,593	
Contract Support					
SAIC-NCI	Dr. Hannon	01/01/07	06/30/08	250,000	*
Research Support	Dr. Chklovskii	07/08/01	06/30/09	289,291	
	Dr. Cline	12/01/04	11/30/09	580,461	
	Dr. Dubnau	09/15/04	06/30/09	346,786	
	Dr. Enikolopov	07/01/07	06/30/09	226,463	*
	Dr. Hannon	09/01/05	08/31/09	330,161	
	Dr. Huang	07/15/05	04/30/09	371,658	
	Dr. Joshua-Tor	07/01/07	06/30/12	402,438	*
	Dr. Joshua-Tor	07/01/05	06/30/09	282,059	
	Dr. Joshua-Tor	02/01/06	01/31/10	293,750	
	Dr. Krainer	09/21/07	08/31/11	620,984	*
	Dr. Lowe	07/01/99	07/31/09	405,752	
	Dr. Malinow	04/01/95	02/29/08	575,356	
	Dr. Martienssen	08/01/07	07/31/11	428,531	*
	Dr. Mills	12/26/07	11/30/12	314,922	*
	Dr. Mitra	03/04/05	02/28/09	477,978	
	Dr. Mittal	04/01/04	02/28/09	296,523	
	Dr. S. Muthuswamy	03/01/06	02/29/08	83,246	
	Drs. Powers/Lowe	12/02/06	11/30/11	374,754	
	Dr. Sebat	04/01/07	03/31/10	732,400	*
	Dr. Sebat	09/30/05	07/31/10	405,328	
	Dr. Skowronski	04/01/98	04/30/08	664,320	

*New grants awarded in 2007

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2007 Funding*	
	Dr. D. Spector	04/01/07	03/31/11	662,242	*
	Dr. D. Spector	09/01/04	08/31/08	310,885	
	Dr. Stein	05/04/07	03/31/11	1,275,000	*
	Dr. Stein	09/25/06	06/30/09	407,213	
	Dr. Stein	04/01/07	02/28/11	1,003,650	*
	Dr. Stenlund	07/01/07	06/30/08	419,375	*
	Dr. Stillman	07/01/91	05/31/08	559,923	
	Dr. Svoboda	12/04/98	11/30/08	290,223	
	Dr. Svoboda	06/01/03	03/31/08	281,255	
	Dr. Svoboda	03/01/04	02/28/09	325,452	
	Dr. Tansey	05/01/07	04/30/11	377,250	*
	Dr. Tonks	07/01/05	06/30/09	369,649	
	Dr. Tonks	07/21/06	05/31/10	289,122	
	Dr. Tully	03/01/03	02/29/08	361,613	
	Dr. Van Aelst	05/01/03	04/30/08	321,835	
	Dr. Zador	01/23/03	12/31/07	401,791	
	Dr. Zhang	08/13/07	07/31/11	318,804	*
	Dr. Zhong	07/01/03	06/30/08	329,067	
Research Subcontracts					
NIH/Booz Allen Hamilton caBIG Consortium Agreement	Dr. Stein	03/07/07	03/06/08	14,968	
NIH/Brookhaven National Laboratory Consortium Agreement	Dr. Stillman	09/01/06	08/31/11	36,624	
NIH/Caltech Consortium Agreement	Dr. Stein	09/01/03	08/31/08	545,266	
NIH/Caltech Consortium Agreement	Dr. Svoboda	04/04/03	02/29/08	144,637	
NIH/Columbia University Consortium Agreement	Dr. Lowe	08/18/06	07/31/11	415,385	
NIH/Columbia University Consortium Agreement	Drs. Wigler/Sebat	09/01/06	05/31/10	286,067	
NIH/Georgia Institute of Technology Consortium Agreement	Dr. D. Spector	09/30/06	09/29/11	140,000	
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Van Aelst	07/10/03	06/30/08	338,319	
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Mittal	04/01/04	03/31/09	137,498	
NIH/Memorial Sloan-Kettering Consortium Agreement	Dr. Hannon	05/04/07	03/31/11	114,738	*
NIH/Oxford University Consortium Agreement	Dr. Stein	03/01/06	02/28/11	157,509	
NIH/Rutgers University Consortium Agreement	Dr. Mitra	12/08/04	11/30/08	107,916	
NIH/University of California–Berkeley Consortium Agreement	Dr. Stein	09/01/07	08/31/10	67,800	*
Fellowship Support	Dr. Chicas	04/01/06	03/31/09	52,048	
	Dr. Demas	02/16/07	02/15/10	49,646	*
	Dr. Slotkin	09/15/06	09/14/09	45,976	
	Dr. Yang	03/01/06	02/28/09	52,048	
Career Development Support	Dr. Silva	04/01/07	03/31/08	112,383	*
Graduate Training Support	Dr. Joshua-Tor/Watson School of Biological Sciences	07/01/02	06/30/12	205,229	
Course Support	X-Ray Methods in Structural Biology	07/01/07	06/30/12	69,935	*
	Immunocytochemistry, In Situ Hybridization, and Live Cell Imaging	07/01/98	08/31/10	81,983	

*New grants awarded in 2007

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2007 Funding*	
	Programming for Biology	07/07/00	08/31/09	55,168	
	C. elegans	08/01/98	08/31/11	76,335	
	Integrated Data Analysis for High-throughput Biology	05/01/07	04/30/08	70,860	*
	Neurobiology of *Drosophilia*	07/01/01	06/30/12	66,552	
	Advanced Techniques in Molecular Neuroscience	07/01/01	06/30/12	75,438	
	Imaging Structure and Function in the Nervous System	07/01/01	06/30/12	82,399	
	Cell and Developmental Biology of *Xenopus*	04/01/93	03/31/09	21,243	
	Protein Purification and Characterization	01/01/83	03/31/11	86,000	
	Molecular Embryology of the Mouse	01/01/83	03/31/11	98,000	
	Eukaryotic Gene Expression	01/01/83	03/31/11	97,836	
	Proteomics	07/01/03	06/30/11	74,192	
	Yeast Genetics and Genomics	07/01/07	05/31/10	47,500	*
Meeting Support	Microbial Pathogenesis and Host Response	05/15/03	04/30/08	13,787	
	Eukaryotic mRNA Processing	08/01/03	07/31/08	6,000	
	The Ubiquitin Family	04/11/07	03/31/12	5,000	*
	The Biology of Genomes	04/08/05	03/31/08	31,388	
	Phosphorylation, Signaling, and Disease	05/01/07	04/30/08	4,000	*
	Rat Genomics and Models	09/30/05	08/31/08	15,000	
	Receptors, Channels, Synapses	04/01/06	03/31/11	29,475	
	72nd Symposium: Clocks and Rhythms	04/01/07	03/31/08	10,000	*
	Telomeres and Telomerase	05/01/07	04/30/08	20,000	*
	Neurobiology of *Drosophilia*	07/01/07	06/30/08	10,000	*
	Mechanisms of Eukaryotic Transcription	07/01/07	06/30/08	3,000	*
	Genome Informatics	08/02/07	07/31/08	22,000	*
	Cell Death	09/14/07	08/31/12	5,000	*
NATIONAL SCIENCE FOUNDATION					
Multiple Project Award Support	Dr. Hannon	09/01/06	08/31/09	400,000	
	Dr. Jackson	07/01/05	06/30/10	1,002,269	
	Dr. McCombie	09/01/06	08/31/08	1,130,076	
	Drs. Stein/Ware	10/01/07	09/30/11	1,589,300	*
	Dr. Stein	12/15/03	11/30/08	1,580,029	
Research Support	Dr. Jackson	04/01/07	03/31/10	140,000	*
	Dr. Lukowitz	04/01/05	03/31/08	129,260	
	Dr. Timmermans	09/01/06	08/31/09	130,000	
	Dr. Timmermans	09/01/06	08/31/09	164,319	
	Dr. Van Aelst	08/15/05	07/31/08	135,266	
	Dr. Ware	09/01/03	08/31/08	276,273	
	Dr. Zador	10/01/07	09/30/09	10,751	*
	Dr. Zhang	09/15/03	08/31/08	91,000	
Research Subcontracts					
NSF/Cornell University Consortium Agreement	Dr. Timmermans	09/01/06	08/31/08	103,520	*
NSF/Cornell University Consortium Agreement	Dr. Ware	11/01/06	10/31/08	150,000	*
NSF/National Center for Genome Research Consortium Agreement	Dr. Stein	09/01/05	08/31/08	57,816	
NSF/New York University Consortium Agreement	Drs. McCombie/Martienssen	10/01/04	09/30/09	257,992	
NSF/North Carolina State University Consortium Agreement	Dr. Martienssen	10/01/04	09/30/09	185,892	

*New grants awarded in 2007

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2007 Funding*	
NSF/University of Arizona Consortium Agreement	Dr. Stein	10/01/06	09/30/08	152,775	
NSF/University of California–Berkeley Consortium Agreement	Dr. Jackson	08/01/06	07/31/11	209,119	
NSF/University of California–Davis Consortium Agreement	Dr. Martienssen	09/01/06	08/31/10	258,944	
NSF/University of Florida Consortium Agreement	Dr. Martienssen	08/01/07	07/31/09	97,991	*
NSF/University of Wisconsin Consortium Agreement	Drs. Stein/Ware	01/01/04	12/31/08	214,457	
NSF/Washington University Consortium Agreement	Drs. McCombie/Martienssen/Stein/Ware	11/15/05	10/31/08	1,432,423	
Fellowship Support	C. Malone	06/01/07	05/31/08	40,500	*
Undergraduate Training Support	Dr. Clark/Watson School of Biological Sciences	04/15/05	03/31/08	117,473	
Meeting Support	Plant Genomes	03/01/07	02/29/08	14,880	*
	Eukaryotic mRNA Processing	08/15/07	07/31/08	3,000	*
	Neurobiology of *Drosophilia*	07/01/07	06/30/08	12,713	*
	Eukaryotic DNA Replication	08/15/07	07/31/08	3,000	*
	Mechanisms of Eukaryotic Transcription	08/15/07	07/31/08	3,000	*
Course Support	Molecular Techniques in Plant Science	07/01/07	06/30/08	85,680	*
	Advanced Bacterial Genetics	07/15/04	06/30/09	77,450	
	Cell and Developmental Biology of *Xenopus*	05/01/07	04/30/10	20,000	*
UNITED STATES DEPARTMENT OF AGRICULTURE					
Research Support	Dr. Jackson	09/01/05	08/31/08	120,224	
	Dr. McCombie	09/15/04	09/14/09	382,425	
	Dr. Stein	09/22/03	09/14/08	244,953	
	Dr. Timmermans	09/15/06	09/14/09	133,359	
Fellowship Support	Dr. Whipple	09/15/06	09/14/08	63,181	
UNITED STATES DEPARTMENT OF THE ARMY					
Research Support	Dr. Hannon	02/01/06	01/31/09	169,500	
	Dr. Hannon	02/15/06	02/14/09	169,500	
	Dr. Lazebnik	12/01/06	11/30/09	210,000	
	Drs. Lucito/Tonks	08/01/07	07/31/08	251,750	*
	Dr. Mu	07/01/07	06/30/10	167,500	*
	Dr. Wigler	04/01/04	08/31/08	1,378,250	
	Dr. Zhong	12/15/04	12/14/08	334,511	
Fellowship Support	X. He	07/16/07	02/15/08	17,500	
	D. Khalil	12/01/06	11/30/09	33,300	
	E. Murchison	02/16/05	07/15/07	12,500	
	A. Rosenberg	02/28/05	02/28/08	30,000	
	D. Simpson	09/01/06	08/31/09	32,400	

*New grants awarded in 2007

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2007 Funding*
Research Subcontracts			
U.S. Army/New York University School of Medicine Consortium Agreement	Dr. Lucito	09/27/04 09/26/09	124,266
UNITED STATES DEPARTMENT OF ENERGY			
Research Subcontracts			
DOE/Texas A&M Consortium Agreement	Dr. Ware	09/15/06 09/14/08	39,528 *
MISCELLANEOUS SOURCES OF FUNDING			
Equipment Support			
Breast Cancer Help Inc.	Dr. Sordella	02/01/07 01/31/08	7,337 *
Elaine Hayes Special Effects Salon	Dr. S. Muthuswamy	07/01/07 06/30/08	20,000 *
F.M. Kirby Foundation, Inc.	Dr. Turner	10/01/07 09/30/08	75,000 *
OSIA NYS General Lodge Foundation Inc.	Dr. Sordella	02/01/07 01/31/08	2,000 *
W.I. Youth Enrichment Serv. Inc.	Dr. Sordella	02/01/07 01/31/08	5,337 *
Program Project Support			
The Leukemia & Lymphoma Society	Dr. Lowe	10/01/03 09/30/08	1,250,000
Pioneer Hi-Bred International, Inc.	Drs. Jackson/Lukowitz/Martienssen/ Timmermans/Ware	07/01/07 06/30/12	1,600,000 *
Theodore R. and Vada S. Stanley	Drs. Watson/McCombie/Sebat	07/01/07 06/30/12	5,000,000 *
Research Support			
American Cancer Society	Dr. Wigler	01/01/07 12/31/07	10,000 *
American Cancer Society	Dr. Wigler	01/01/06 12/31/10	60,000
American Cancer Society	Dr. Mills	07/01/06 06/30/10	240,000
Anonymous Gift	Dr. Malinow	09/01/06 08/31/08	130,000
Anonymous Gift	Dr. Sebat	09/01/07 08/31/09	248,689 *
Anonymous Gift	Dr. Zador	09/01/07 08/31/09	500,000 *
Ashner Family Evergreen Foundation	Dr. Mitra	04/01/07 03/31/08	9,000 *
Autism Speaks, Inc.	Dr. Zador	07/01/07 06/30/10	150,000 *
Mr. and Mrs. Donald E. Axinn	Drs. Lowe/Hannon	2007	250,000 *
Brain Tumor Society	Dr. Hannon	11/01/06 10/31/08	100,000
Breast Cancer Help Inc.	Dr. S. Muthuswamy	07/01/07 06/30/08	7,337 *
Breast Cancer Research Foundation	Dr. Wigler	10/01/07 09/30/08	250,000 *
Clear Channel Worldwide (Walk 97.5 FM) Breast Cancer Research Walk	Dr. S. Muthuswamy	04/01/07 03/31/08	4,000 *
Coleman Fung Foundation Inc.	Dr. Zador	02/01/07 01/31/10	50,000 *
Mr. and Mrs. James Cook	Dr. Lazebnik	04/01/07 03/31/08	2,500 *
Mrs. Shelby Cullom Davis	Dr. Hannon	01/15/07 01/14/08	1,600,000 *
Eisai Co., Ltd.	Dr. Malinow	10/01/07 09/30/08	10,000 *
The Eppley Foundation for Research, Inc.	Dr. Enikolopov	07/01/07 06/30/08	35,000 *
Find A Cure Today (F.A.C.T.)	Dr. S. Muthuswamy	12/01/07 11/30/08	30,000 *
FSMA	Dr. Hastings	01/15/06 08/31/07	70,444
Genentech, Inc.	Dr. Hannon	04/24/07 04/23/08	500,000 *
The Ryan Gibson Foundation	Dr. M. Spector	08/01/07 07/31/08	50,000 *
The Joni Gladowsky Breast Cancer Foundation	Dr. S. Muthuswamy	12/01/07 11/30/08	32,678 *
GlaxoSmithKline	Dr. Powers	09/20/06 09/19/08	484,476
GlaxoSmithKline	Dr. S. Muthuswamy	10/01/06 09/30/08	168,024
Glen Cove C.A.R.E.S., Inc.	Dr. S. Muthuswamy	04/01/07 03/31/08	2,000 *
Robert I. Goldman Foundation	Dr. Mittal	12/31/07 12/30/08	30,000 *
Dr. Leo A. Guthart	Drs. Lowe/Hannon	2007	250,000 *
Irving A. Hansen Memorial Foundation	Dr. Tansey	08/01/07 07/31/08	20,000 *
The Thomas Hartman Foundation	Dr. Tonks	07/01/07 06/30/09	172,518 *
Ira Hazan	Dr. Enikolopov	12/01/07 11/30/08	200,000 *

*New grants awarded in 2007

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2007 Funding*	
The Lita Annenberg Hazen Foundation	Dr. Powers	11/01/07	10/31/08	250,000	*
Hearts for Cancer	Dr. S. Muthuswamy	12/01/07	11/30/08	5,830	*
Hope for Depression Research Foundation, Inc.	Dr. Enikolopov	01/01/07	12/31/07	110,000	*
Human Frontier Science Program Organization	Dr. Dubnau	10/01/06	09/30/09	109,980	
Islip Breast Cancer Coalition Inc.	Dr. S. Muthuswamy	09/01/07	08/31/08	25,000	*
Joan's Legacy: The Joan Scarangello Foundation to Conquer Lung Cancer	Dr. Sordella	12/01/07	11/30/09	50,000	*
V. Kann Rasmussen Foundation	Dr. Trotman	09/01/07	08/31/12	50,000	*
The Karches Foundation	Dr. Wigler	07/01/05	06/30/10	466,147	
W.M. Keck Foundation	Dr. Mitra	07/01/06	06/30/09	500,000	
The Charles H. Leach, II Foundation	Dr. Enikolopov	03/01/07	02/29/08	2,000	*
Lehman Brothers Foundation	Dr. Trotman	11/01/07	10/31/08	25,000	*
L.I.A.B.C.	Dr. Wigler	2007		27,018	*
Margaret and Richard Lipmanson Foundation	Dr. Furukawa	07/01/07	06/30/08	50,000	*
Little Louie Foundation	Dr. Van Aelst	05/01/07	04/30/08	10,000	*
Long Beach Breast Cancer Coalition	Dr. S. Muthuswamy	03/01/07	02/29/08	3,000	*
Long Island 2-Day Walk to Fight Breast Cancer, Inc.	Dr. S. Muthuswamy	08/01/07	07/31/08	29,000	*
Manhasset Women's Coalition Against Breast Cancer	Dr. Powers	01/01/07	12/31/08	85,000	*
Manhasset Women's Coalition Against Breast Cancer	Dr. S. Muthuswamy	01/01/07	12/31/07	15,000	*
Manyu Ogale Fund	Dr. Stillman	10/01/07	09/30/08	505,000	*
The G. Harold and Leila Y. Mathers Charitable Foundation	Dr. Huang	07/01/07	06/30/10	194,093	*
The Elizabeth McFarland Breast Cancer Fund	Dr. Wigler	12/01/07	11/30/08	67,158	*
The Don Monti Memorial Research Foundation	Dr. Lowe	06/01/06	12/31/07	400,000	*
The Don Monti Memorial Research Foundation	Dr. Lowe	09/01/07	08/31/08	150,000	*
Louis Morin Charitable Trust	Dr. D. Spector	12/01/07	11/30/08	35,000	*
Louis Morin Charitable Trust	Dr. Zador	12/01/07	11/30/08	35,000	*
Muscular Dystrophy Association	Dr. Krainer	07/01/07	06/30/10	254,000	*
NARSAD	Dr. Enikolopov	09/15/06	09/14/08	50,000	
NYSTAR	Dr. Paddison	03/01/07	02/28/09	115,957	*
Philip Morris USA	Dr. Mittal	07/01/07	06/30/08	423,750	*
Philips Research North America	Dr. Lucito	04/01/07	03/31/09	274,288	*
Philips Research North America	Dr. Wigler	04/01/07	03/31/09	322,534	*
Diane Emdin Sachs Memorial Fund	Dr. Mu	12/01/07	11/30/08	31,114	*
The Simons Foundation	Dr. Mills	01/01/07	12/31/09	462,968	*
The Simons Foundation	Dr. Huang	10/01/07	09/30/10	423,853	*
Debra P. Sondock	Dr. Lazebnik	04/01/07	03/31/08	10,000	*
Starr Cancer Consortium	Dr. Hannon	11/01/07	10/31/09	480,000	*
Starr Cancer Consortium	Dr. Krainer	11/01/07	10/31/09	300,000	*
Starr Cancer Consortium	Dr. Powers	11/01/07	10/31/09	378,343	*
Starr Cancer Consortium	Dr. Van Aelst	11/01/07	10/31/09	240,000	*
Starr Cancer Consortium	Dr. Wigler	11/01/07	10/31/09	120,000	*
Starr Cancer Consortium	Drs. Powers/Lucito	11/01/07	10/31/09	300,000	*
Women in Science	Dr. D. Spector	01/01/07	12/31/07	66,275	*
Women's Insurance Network of Long Island	Dr. S. Muthuswamy	06/01/07	05/31/08	16,951	*

*New grants awarded in 2007

Grantor	*Program/Principal Investigator*	*Duration of Grant*	*2007 Funding*
Fellowship Support			
The Rita Allen Foundation	Dr. Trotman	09/01/07 08/31/10	50,000 *
American Cancer Society	Dr. Karginov	01/01/07 12/31/09	44,000 *
Arnold and Mabel Beckman Foundation	Watson School of Biological Sciences	09/01/05 08/31/10	350,000
Arnold and Mabel Beckman Foundation	Dr. Dubnau	09/01/05 08/31/08	88,000
Terri Brodeur Breast Cancer Foundation	Dr. Chatterjee	01/01/07 12/31/08	50,000 *
C.J. Martin Fellowship	Dr. Irvine	04/01/06 03/31/10	45,966
Carnegie Institution	Dr. Timmermans	09/01/07 08/31/08	15,385 *
Cashin Family Fund	Watson School of Biological Sciences	09/01/04 08/31/08	40,000
The Mary K. Chapman Foundation	Dr. Stillman	12/01/07 11/30/08	85,000 *
CSHL Association Fellowship	New Investigator Support	2007	280,000 *
The Danish Cancer Society	Dr. Jensen	01/01/07 12/31/09	71,312 *
DFG (German Reasearch Council)	Dr. Miething	01/01/07 12/31/08	55,000 *
Ellison Medical Foundation	Dr. Chien	07/01/07 06/30/09	49,250 *
Francis Goelet Trust	Dr. Wigler	10/01/05 09/30/08	64,028
The Allen and Lola Goldring Foundation	Dr. Stillman	09/01/07 08/31/08	75,000 *
Heat and Stroke Foundation	Dr. Boivin	07/01/06 06/30/08	38,870
Human Frontier Science Program Organization	Dr. Kasri	09/01/06 08/31/09	38,000
Human Frontier Science Program Organization	Dr. Schlach	08/01/07 07/31/08	38,000 *
Annette Kade Charitable Trust	Watson School of Biological Sciences	03/01/07 02/28/09	50,000 *
Harvey L. Karp Foundation	Dr. Krainer	04/01/07 03/31/08	50,000 *
The Leukemia & Lymphoma Society	Dr. Krizhanovsky	07/01/06 06/30/09	50,000
The Lymphoma Research Foundation	Dr. Kurland	07/01/06 06/30/08	55,000
Merck Career Development Award	Watson School of Biological Sciences	01/01/07 12/31/07	20,000 *
Sir Keith Murdock	Dr. Murchison	04/01/07 09/14/07	25,000 *
NARSAD	Dr. Real	07/01/06 06/30/08	30,000
NARSAD	Dr. Kuhlman	07/01/06 06/30/08	30,000
NARSAD	Dr. Encinas	07/01/07 06/30/09	30,000 *
NYS Department of Health, Health Research Science Board, Breast Cancer Research, and Education Program	Dr. Chen	01/01/06 03/31/08	60,000
The Robert Leet and Clara Guthrie Patterson Trust	Dr. Lima	01/15/07 01/14/09	46,000 *
The Sass Foundation for Medical Research, Inc.	Dr. Stillman	12/01/07 11/30/09	75,000 *
The Seraph Foundation, Inc.	Watson School of Biological Sciences	10/01/04 09/30/08	45,000
Lauri Strauss Leukemia Foundation	Dr. Pardee	02/01/07 01/31/08	15,000 *
Swartz Foundation	Drs. Koulakov/Tsigankov	01/01/07 12/31/07	50,000 *
Swartz Foundation	Dr. Mainen	01/01/07 12/31/07	333,333 *
Swartz Foundation	Drs. Mainen/Felsen	01/01/07 12/31/07	50,000 *
Swartz Foundation	Drs. Zador/Jaramillo	01/01/07 12/31/07	50,000 *
Swartz Foundation	Dr. Zador/Neuro Seminar Support	01/01/07 12/31/07	15,000 *
Swartz Foundation	Drs. Zador/Oswald-Pisarski	01/01/07 12/31/07	16,667 *
Uehara Memorial Foundation, Japan	Dr. Kawakami	01/16/07 01/15/08	33,157 *
Vicom Computer Services, Inc./ IBM Fellowship	Dr. Townsend	02/01/07 12/31/08	225,000 *
Michel David-Weill	Dr. Cline	09/01/06 08/31/09	200,000
Training Support			
Cornelius N. Bliss Memorial Fund	Undergraduate Research Program	2007	4,500 *
Howard Hughes Medical Institute	Undergraduate Research Program	2007	7,000 *
Hunter College	Undergraduate Research Program	2007	7,000 *
Steamboat Foundation	Undergraduate Research Program	05/01/07 04/30/08	12,000 *
William Townsend Porter Foundation	Undergraduate Research Program	04/01/07 03/31/08	10,000 *
John Xue	Undergraduate Research Program	2007	500 *

*New grants awarded in 2007

Grantor	*Program/Principal Investigator*	*Duration of Grant*		*2007 Funding*	
Course Support					
Applied Biosystems	Next-generation Sequencing	08/01/07	07/31/08	25,000	*
Autism Speaks, Inc.	Workshop on Autism Spectrum Disorders	05/01/07	04/30/11	15,000	*
Duke University Medical Center	Infectious Disease: A Challenge for Biomedical Informatics	09/01/07	08/31/08	40,102	*
Howard Hughes Medical Institute	Neurobiology Course Support	01/01/91	12/31/09	750,000	
Illumina, Inc.	Next-generation Sequencing	08/01/07	07/31/08	25,000	*
The Nancy Lurie Marks Family Foundation	Workshop on Autism Spectrum Disorders	05/01/07	04/30/11	15,000	*
Roche Diagnostics	Next-generation Sequencing	08/01/07	07/31/08	25,000	*
The Simons Foundation	Workshop on Autism Spectrum Disorders	05/01/07	04/30/11	15,000	*
Meeting Support					
Boston Biochem, Inc.	The Ubiquitin Family	04/01/07	03/31/08	500	*
Burroughs Wellcome	Microbial Pathogenesis and Host Response	07/01/07	06/30/08	12,000	*
Foundation for the National Institutes of Health	Clinical Cardiovascular Genomics	03/01/07	02/29/08	18,710	*
454 Life Sciences	Workshop on Honey Bee Genomics and Biology	01/01/07	12/31/07	3,000	*
International Business Machines Corporation	Computational Cell Biology	03/01/07	02/29/08	2,000	*
International Vaccine Institute	PDVI (Pediatric Dengue Vaccine Initiative) Research Network Meeting	04/01/07	03/31/08	87,744	*
Roche Applied Science	The Biology of Genomes	03/01/07	02/29/08	5,000	*
Roche Applied Science	Workshop on Honey Bee Genomics and Biology	03/01/07	02/29/08	5,000	*
Solexa, Inc.	The Biology of Genomes	03/01/07	02/29/08	2,500	*
Syngenta	Systems Biology: Global Regulation of Gene Expression	03/01/07	02/29/08	2,000	*
University of Connecticut Health Center	Computational Cell Biology	03/01/07	02/28/09	48,000	*
Library Support					
Richard Lounsbery Foundation, Inc.	Dr. Pollock	06/01/07	05/31/08	5,000	*

*New grants awarded in 2007

DOLAN DNA LEARNING CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2007 Funding*
FEDERAL GRANTS			
National Institutes of Health	Science Education Partnership Award: Nationwide Dissemination of *Inside Cancer* Internet Site	08/07–07/09	$ 19,753
National Science Foundation	Course, Curriculum, and Laboratory Instruction Progam: Nationwide Dissemination of RNAi Curriculum	09/07–08/09	10,973
National Science Foundation	Plant Genome Initiative Educational Outreach: Minority Fellows and Regional Plant Genomics Footlockers	09/04–08/07	58,517
National Science Foundation	Plant Genome Initiative Educational Outreach: Construction and Nationwide Dissemination of *Dynamic Gene* Internet Site	12/05–11/07	141,459
AAAS/NSF	National Science Digital Library: Meta-tagging DNALC Internet Content for BiosciEdNet	10/05–09/09	50,868
Washington University/NSF	Plant Genome Initiative Educational Outreach: Multimedia Materials on Maize Genome Sequencing	11/05–10/08	18,337
Cornell University/NSF	Plant Genome Initiative Educational Outreach: Minority Fellows and Regional Plant Genomics Footlockers	09/05–08/08	52,382
USDA	Systematic Determination of the Maize Gene Set: Educational Outreach	02/06–01/07	3,178
NONFEDERAL GRANTS			
Amgen Foundation	Amgen Leadership Symposium	04/05–12/08	$ 85,233
Clemson University	DNALC Licensing	2007	50,000
Dana Foundation	*Genes to Cognition* (*G2C*) *Online* Internet Site Development	10/04–09/08	239,986
Dialog Gentechnik	DNALC Licensing	2007	24,975
Goldman Sachs Foundation	*Harlem DNA Lab* Planning	2007	50,000
Hewlett Foundation	*Genes to Cognition* (*G2C*) *Online* Internet Site Evaluation	10/05–10/09	68,983
Howard Hughes Medical Institute	Pre-College Science Education Initiative: NYC Teacher Training	09/07–08/08	21,878
North Shore–LIJ Health System	DNALC *West* Operating Support	2007	50,000
Porter Foundation	Scholarships for Minority and Disadvantaged Students	2007	30,000

The following schools each contributed $1000 or more for participation in the *Curriculum Study* Program:

Bellmore–Merrick Central High School District	$1,250	North Shore Central School District	$1,250
Fordham Preparatory School	1,250	North Shore Hebrew Academy	1,500
Garden City Union Free School District	1,250	Oceanside Union Free School District	1,500
The Green Vale School	2,750	Oyster Bay–East Norwich School District	1,250
Half Hollow Hills Central School District	1,250	Plainedge Union Free School District	1,500
Harborfields Central School District	1,500	Plainview–Old Bethpage Central School District	1,250
Huntington Union Free School District	1,250	Portledge School	1,500
Island Trees Union Free School District	1,500	Port Washington Union Free School District	1,500
Jericho Union Free School District	1,500	Ramaz School	2,750
Lawrence Union Free School District	1,250	Roslyn Union Free School District	2,750
Levittown Union Free School District	2,750	South Huntington Union Free School District	1,500
Locust Valley Central School District	1,500	Syosset Central School District	2,750
Massapequa Union Free School District	1,500	Yeshiva University High School for Girls	1,500

The following schools each contributed $1000 or more for participation in the *Genetics as a Model for Whole Learning* Program:

Allen Christian School	$ 1,150	Locust Valley Central School District	$ 8,587
Bay Shore Union Free School District	4,675	Mamaroneck Union Free School District	3,600
Bellmore Union Free School District	5,400	Mattituck–Cutchogue Union Free School District	1,375
Bellmore–Merrick Central School District	16,600	Merrick Union Free School District	1,200
Bethpage Union Free School District	1,950	Mott Hall V Middle School	2,000
Deer Park Union Free School District	1,080	North Bellmore Union Free School District	2,750
Eastern Middle School	4,950	Northport–East Northport Union Free School District	1,187
East Meadow Union Free School District	2,297	Oceanside Union Free School District	1,125
East Williston Union Free School District	2,700	Old Westbury School of the Holy Child	5,575
Elwood Union Free School District	3,820	Oyster Bay–East Norwich Central School District	2,515
Floral Park–Bellerose Union Free School District	5,400	Park City Prep Charter School	1,350
Friends Academy	2,550	Plainview–Old Bethpage Central School District	3,830
Garden City Union Free School District	7,005	Port Washington Union Free School District	5,350
Great Neck Union Free School District	13,380	Roberto Clemente Middle School	1,000
The Green Vale School	1,200	Rockville Centre Union Free School District	11,100
Half Hollow Hills Central School District	7,125	Scarsdale Union Free School District	1,325
Herricks Union Free School District	1,650	St. Dominic Elementary School	3,900
Huntington Union Free School District	6,925	St. Edward the Confessor School	1,820
Jericho Union Free School District	15,625	Syosset Central School District	29,625
Lawrence Union Free School District	14,100	Yeshiva Darchei Torah	1,900

BANBURY CENTER GRANTS

Grantor	*Program*	*Duration of Grant*	*2007 Funding*
FEDERAL SUPPORT			
Centers for Disease Control and Prevention (CDC)	The Laboratory Diagnosis of Lyme Disease II	2007	$ 8,900*
NIH–National Institute of Allergy & Infectious Diseases	The Laboratory Diagnosis of Lyme Disease II	2007	17,100*
NIH–National Institute of Mental Health (through a grant to University of Illinois)	Fragile-X Syndrome and Mechanisms of Synaptic Translation	2007	44,564*
The National Science Foundation	Design Principles in Biological Systems–2	2007	47,830*
The National Science Foundation	Second Environment Ontology Workshop	2007	30,000
U.S. Department of Homeland Security	Microbial Forensics: Enduring Research Pathways	2007	9,800*
NONFEDERAL SUPPORT			
Meeting Support			
AstraZeneca Pharmaceuticals	Neurobiology of Depression: From Molecules to Mood	2007	5,000*
Boehringer Ingelheim Fonds Foundation for Basic Research in Medicine	Science—Get It Across!	2007	38,461*
The Champalimaud Foundation	Champalimaud Foundation: Neuroscience and Cancer Research	2007	108,035*
Greater NY Chapter of the ALS Association	Drug Discovery, Biomarkers, and Clinical Trials for ALS	2007	44,787*
The W.M. Keck Foundation	The Brain Architecture Project Annual Meeting	2007	23,964*
Eli Lilly & Company	Neurobiology of Depression: From Molecules to Mood	2007	2,000*
Marsh & McLennan Companies–MMC Matching Gifts to Education Program	Interdisciplinary Memory Symposium in Neurosciences and the Humanities	2007	5,000*
Memory Pharmaceutical Corp.	Neurobiology of Depression	2007	5,000*
Haig R. Nalbantian	Interdisciplinary Memory Symposium in Neurosciences and the Humanities	2007	5,000*
Open Biosystems	Interactome Mapping Project for Human and Model Organisms	2007	3,000*
OSI Pharmaceuticals, Inc.	Epithelial Mesenchymal Transition	2007	19,748*
Mr. and Mrs. Howard Phipps, Jr.	Interdisciplinary Memory Symposium in Neurosciences and the Humanities	2007	10,000*
Private funding	Cell Transplantation as a Therapy for Parkinson's Disease	2007	45,944*
Private funding	Retreat From Reason	2007	17,915*
Roche Pharmaceuticals	Neurobiology of Depression: From Molecules to Mood	2007	10,000*
The Daniel and Joanna S. Rose Fund, Inc.	Interdisciplinary Memory Symposium in Neurosciences and the Humanities	2007	10,000*
Albert B. Sabin Vaccine Institute (with support of the Robert Wood Johnson Foundation)	Protecting Public Trust in Immunization	2007	26,093*
The Satenik and Adom Ourian Educational Foundation	Interdisciplinary Memory Symposium in Neurosciences and the Humanities	2007	3,000*
The Selz Foundation, Inc.	Interdisciplinary Memory Symposium in Neurosciences and the Humanities	2007	1,000*
Sepracor	Neurobiology of Depression: From Molecules to Mood	2007	5,000*
Alfred P. Sloan Foundation	Using Bar-code Data in Studies of Molecular and Evolutionary Dynamics	2007	45,000*
The Swartz Foundation	New Frontiers in Studies of Nonconscious Processing	2007	35,956*
University of Georgia (with support of Arborgen, Canadian Forest Service, European Union Evoltree, Genome British Columbia, Genome Canada, Oregon State University, Port Blakeley Tree Farms, Starker Forests, University of California at Davis, University of Maine, and USDA Forest Service)	International Workshop on Conifer Genomics	2007	27,139*
Wyeth Pharmaceuticals	Neurobiology of Depression: From Molecules to Mood	2007	5,000*

*New grants awarded in 2007

DEVELOPMENT

It has been just over 1 year since the public announcement of Cold Spring Harbor Laboratory's $200 million capital campaign. In that time, we have seen tremendous progress in the construction of six new buildings, which now can clearly be seen from across the harbor. Work is currently focused on the interior portions of each building. Fund-raising for the campaign has been equally successful, with more than $20 million raised since last year and over $150 million raised toward the goal.

In 2007, the Laboratory received a $25 million grant to support schizophrenia research, one of the largest gifts in our history. This wonderful commitment from Vada and Ted Stanley has given our researchers the necessary resources to tackle this debilitating disease. The second annual Double Helix Medals Dinner was held on November 8 at the Mandarin Oriental and honored Dr. Michael Wigler, Dr. Richard Axel, and David Koch for their extraordinary contributions to genetic disease research. The event raised over $3 million in unrestricted funding for the Laboratory.

This year's many advances in science could not have happened without the support of our donors. Thank you for your commitment to scientific research and education at Cold Spring Harbor Laboratory.

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. ScienceWatch, an independent ratings service, has ranked the Laboratory's molecular biology and genetics program number one during the last decade. Additionally, *Charity Navigator*, a philanthropic evaluator, has bestowed their highest four-star rating on the Laboratory for 6 consecutive years.

Less than half of the Laboratory's annual revenues are derived from Federal grants and contracts, and we rely heavily on support from foundations, corporations, and individuals. There are a variety of ways to give to the Laboratory:

Capital and Endowment Campaign Support: Cold Spring Harbor has embarked on a $200 million capital and endowment campaign to speed the translation of genetic discoveries into diagnostic and therapeutic treatments and to expand Lab facilities and staff. Endowment gifts can be directed toward supporting cancer, neuroscience, or educational programs. Capital gifts can be made to name laboratories and conference rooms in one of our six new research buildings.

Annual Fund and Research Support: Donations provide funding for some of the most determined and innovative young researchers in science today. Your gift is an investment in some of the world's best research in cancer, neuroscience, plant biology, and bioinformatics.

Science Education: Donations support programs at the Dolan DNA Learning Center and the Watson School of Biological Sciences, where the next generation of scientists learn about genetics in an exciting and interactive environment.

Planned and Estate Gifts: Individuals who inform us of their intention to make a gift to Cold Spring Harbor Laboratory from their estate are invited to become members of The Harbor Society. Estate gifts help to ensure that CSHL will advance its mission for many years to come.

For additional information, please contact the Vice President for Development, Cold Spring Harbor Laboratory, One Bungtown Road, Cold Spring Harbor, New York 11724. Phone number: 516-367-6865.

President's Council

The President's Council is composed of individuals who gather together for stimulating scientific events related to current CSHL research. Their contributions of $25,000 or more annually support research and education at CSHL and help to fund the Cold Spring Harbor Laboratory Fellows, a group of exceptionally talented young scientists who show the capacity for high-level innovative research. Rather than conducting a postdoctoral apprenticeship with a senior scientist, as is conventional, Fellows begin independent research immediately following receipt of their Ph.D.s. This rigorous program promotes outstanding young scientists to advance their careers at a young age so that they can promptly contribute new insights to important biomedical science.

In April, President's Council Members Diahn and Tom McGrath hosted a cocktail reception featuring a talk by Professor Scott Lowe on *Innovation, Integration, and Renewal in Cancer Research at CSHL*. The October President's Council retreat brought together leaders in the field of RNA interference (RNAi), including Nobel laureates Sydney Brenner and Phillip Sharp, as well as CSHL Professors Greg Hannon and Marja Timmermans. President's Council members learned about how the phenomenon of RNAi can turn on and off genes, suggesting exciting new strategies to develop therapies for disease, and had the opportunity to see the results of RNAi firsthand through experiments at the Dolan DNA Learning Center.

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President's Council Co-Chairman Dr. William Haseltine (*left*) with Edward Epstein and David Koch at the April 9 spring reception hosted by Diahn and Tom McGrath.

Kathryn Davis and Dolan DNALC Executive Director Dave Micklos during the fall President's Council event.

Cold Spring Harbor Laboratory Association (CSHLA)

We are grateful to the growing number of members of the Cold Spring Harbor Laboratory Association who encourage young scientists as they endeavor to solve complicated biomedical problems with ambitious approaches that are not yet eligible for government grants. In 2007, our annual fund goal of $1.2 million was surpassed for a total of $1,260,000 raised by nearly 1000 members.

Laboratory Association members receive invitations to social and fund-raising events in support of CSHL, subscriptions to our quarterly magazine *The Harbor Transcript* and our monthly net-letter, and are encouraged to get to know our scientists and help to integrate them and their families into the community.

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Dolan DNA Learning Center Advisory Boards

The Corporate Advisory Board (CAB), established in 1992, serves the Dolan DNA Learning Center as a bridge to the corporate community and assists in securing annual unrestricted funds. Composed of influential business leaders from large and small companies on Long Island and Manhattan, the CAB is a necessary and vital component to the continued success and mission of the DNALC. The Dolan DNA Learning Center Committee oversees the strategic development of the Center, including capital resource development and the evolution of satellite locations in North America and beyond.

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On June 19th, the DNALC held its 14th annual golf tournament at Piping Rock Country Club. Pictured at the event are Don (*left*) and Arthur Saladino.

Dolan DNA Learning Center Corporate Advisory Board Chairman Eddie Chernoff (*seated, right*) with friends at the 2007 golf tournament.

Honor Roll of Donors

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Double Helix Medal recipient David Koch pictured with *(left to right)* CSHL Honorary Trustee Evelyn Lauder, Amanda Haynes-Dale, and Julia Koch.

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Dr. Michael Wigler (*left*) and Dr. Richard Axel (*right*) receive their Double Helix Medals from CSHL Honorary Trustee Evelyn Lauder and President Dr. Bruce Stillman.

CSHL Chairman Eduardo Mestre with his wife Gillian (*center*) and Laboratory Trustee Louise Parent at the Double Helix Medals Dinner.

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CSHL Trustee Jamie Nicholls (*center*) and her husband Fran Biondi are pictured with Sophie Ayres at the Double Helix Medals Dinner. Jamie and Fran were co-chairs of the event.

*Gift or pledge made to the Hillside Campus Campaign 2003–2007

CSHL Chancellor Emeritus Dr. James Watson (*left*) with Laboratory Trustee David Rubenstein at the Double Helix Medals Dinner.

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On October 12, the renovation of the Carnegie building officially began with a groundbreaking ceremony. Pictured (*left to right*) are Library Director Mila Pollock, Dr. Waclaw Szybalski, Dr. James Watson, Laurel Hollow Mayor The Honorable Harry Anand, and Laboratory President Dr. Bruce Stillman.

The 9th Annual *Jazz at the Lab* was held on April 14. Pictured at the event are (*left to right*) CSHL Association Director Lauryl Palatnick, Kerry and Andy Glanz, Rob Palatnick, Elizabeth and Rob Bailenson, and Saul and Lynn Federman.

Lori and Stephen Garofalo
Dr. and Mrs. Charles B. Goodwin
Lola and John Grace
Mr. and Mrs. Oliver R. Grace, Jr.–OBX
Mr. and Mrs. Randall Hack
The Irving A. Hansen Memorial Foundation

On January 28, the CSHL Association held its annual meeting, which featured a talk by Dr. Philip R. Reilly, author of *The Strongest Boy in the World*. Pictured are CSHLA Honorary Director George Cutting and Laboratory Honorary Trustee Mary Lindsay.

Elaine Hayes Special Effects Salon
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*Gift or pledge made to the Hillside Campus Campaign 2003–2007

Pictured (*left to right*) are Michael and Jackie Shapiro, Larry and Jessica Glassman, Nancy and Ron Israeli, and Marcie and Jeff Kaiser at *Jazz at the Lab*.

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Pictured (*left to right*) are Bob Hussey, David Knott, and Tom Gimbel at *Jazz at the Lab*.

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Jason Gladowsky (*left*) with his sister Alison and father Elliott as they present a check from the Joni Gladowsky Foundation to Laboratory scientist Dr. Senthil Muthuswamy. The foundation was started in memory of their mother/wife, Joni, who lost her battle against breast cancer.

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